The Journal of Laboratory and Clinical Medicine

VOL. 19 ST. LOUIS, MO., JUNE, 1934 NO. 9

CLINICAL AND EXPERIMENTAL

INTRACELLULAR STRUCTURES IN MONOCYTES IN CASES OF MALIGNANT DISEASE*

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THE occurrence of bodies of spiral form within the large mononuclear leucocytes in cases of malignant disease was noted in 1916[3] but without arriving at any definite conclusion as to their nature. It was considered possible that their presence might afford a means of diagnosis.

Since that time, a search for bodies of this kind has been made in every subsequent available case, whether hospital or private (in England), and it was found that when they were present, the case was very often one of malignant disease. It was, therefore, concluded that their presence could not be accidental, or a coincidence, but that they must represent genuine structures and have a positive significance. The problem remained as to whether they were possibly mitochondrial in nature, or perhaps belonged to the category of the inclusion bodies described in the case of virus diseases by various observers.

Technical Note.—Ordinary air-dried blood films are used. They are stained either by Pappenheim's panoptic method or by Leishman's stain. The latter is time saving and satisfactory. No other methods of staining fulfill the requirements. A strong light is essential for the examination. The endothelioid monocytes are specially studied. When the filamentous forms are not very distinct they are traceable by searching first for their nodal points.

Forms Noted.—Coccoid and bacillary (batonnet) forms are well known to occur in the mononuclear leucocytes of normal blood and of exudates. They

*From the Department of Surgery, McGill University.
Received for publication, April 24, 1933.
Under a grant for cancer research contributed by an anonymous donor.

vary in size from the ordinary azure granule to the granules and rods known as chondriomes and mitochondria found in various tissue cells. It is not these that form the subject of this communication, but linear forms; namely, (a) irregularly wavy or filamentous lines which may assume a definite spirillar form. These correspond to the chondriocontes of cytology (Fig. 1). (b) Curved or angular lines marked by nodal thickenings. Some of these are definitely flexed at the nodal thickenings and may be called "geniculate bodies." (c) Branching lines or "mycelioid" bodies. There is sometimes a round thickening at one end as shown in Cell 6 of the figure. (d) Other forms include (1) "beaded" forms, which appear as rows of fine dots lying in a linear vacuole. These correspond to

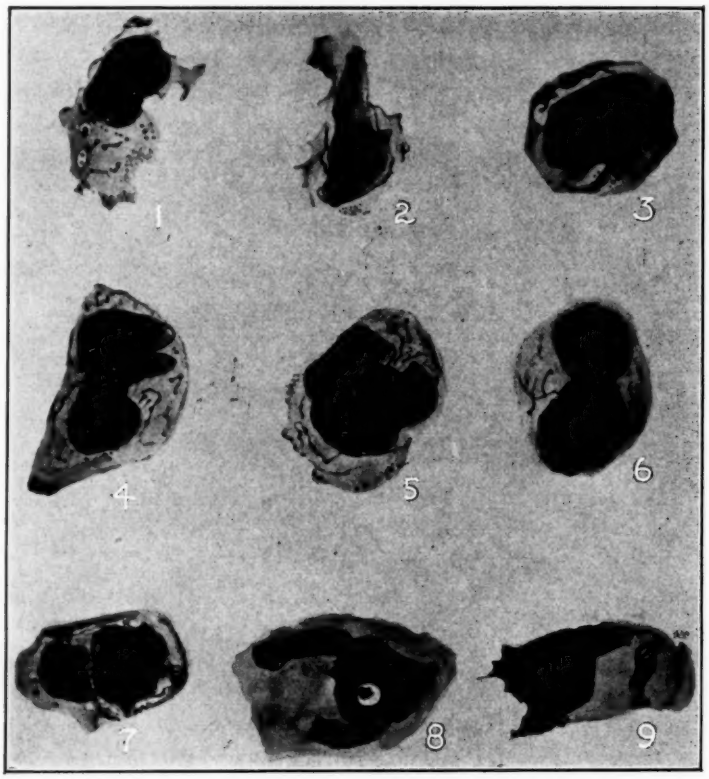

Fig. 1.—Nine cells are shown, each from a different case of malignant disease. Eight show cytoplasmic structures, and one shows an intranuclear body. Magnification: Slightly larger than that apparent with the ordinary oil immersion lens and medium high ocular. Cells 1 and 2 are hyaline leucocytes. The rest are monocytes. *Coccal forms:* Cells 1, 2, 4, 5, and 7 show the ordinary fine azure granules. Cell 1 also shows a single granule in a vacuole and a diploid body in a vacuole. *Undulant and spiral forms* are shown in cells 3, 4, 5, and 7. *Noded or beaded lines* in cells 1, 2, 4, and 5. *Spirillar form* with globular end in cell 2. *Mycelioid form* in cell 6. *Protozoal form* in cell 9. Cell 7 was considered possibly a sarcoma cell.

chondriomites of general cytology, (2) intranuclear bodies in vacuoles, showing a very fine granulation (Cell 8 of figure), (3) larger bodies occasionally occur which might easily be mistaken for protozoa.

Possible Errors of Interpretation.—Spiral bodies may be simulated by edges of cell substance wrinkled by spreading the film, so that an optical effect is produced. Nodal points may be passed by as being simply azure granules, the filaments being overlooked. Azure granules may appear to be connected simply

through an optical effect. Granules in intracytoplasmic clefts may appear as chondriomites. Only forms which appear definitely red or purple red are accepted as genuine intracellular structures.

TABLE I

FREQUENCY WITH WHICH INTRACELLULAR BODIES WERE NOTED IN THE MONONUCLEAR LEUCOCYTES IN PROVED CASES OF MALIGNANT DISEASE
(1925-1933)

SITE (TYPE)	NO. CASES EXAMINED	% POSITIVE	NUMBERS PER 1000 MONONUCLEARS				
			TYPE (A)	(B)	(C)	(D)	TOTAL
Alimentary tract	77	100	84	20	6	22	132
Epithelioma	43	92	71	17	5	35	128
Cervix and uterus	25	90	60	30	9	17	116
Breast	29	100	55	26	7	10	98
Other sites	27	93	76	19	9	28	132
Sarcoma	22	87	70	22	10	11	113
Total untreated patients	223	93	71	21	7	21	120
Patients under radium treatment	21	73	51	24	9	7	91
Patients definitely nonmalignant	435	6.7	5.6	0.2	0.04	2.1	8

Comment.—The third column shows that intracellular structures of the types described occur in a high percentage of cases of carcinoma or sarcoma. Cases are counted as negative if these bodies are not seen within the conditions selected, namely during the scrutiny of between 100 and 200 leucocytes. The last column indicates how soon the bodies will be seen. Thus, in the alimentary cases, 132 out of every 1,000 mononuclear leucocytes showed them; in other words, about every eighth mononuclear.

This serves to show that this form of study can come within the scope of routine work.

As regards the first column, cases in the alimentary tract include all tumors primary in the stomach or onward, that is, not epitheliomas. The term "epithelioma" covers cases which were external or buccal or in the upper alimentary tract as far as the cardiac orifice. The negative cases in this group were mostly esophageal. Sarcomas appear to provide most of the negative results, one case was a myxosarcoma and one a fibrosarcoma; there was one case of fibromyoma turning malignant. Perhaps it is not surprising that these should yield negative results.

The cases described as "definitely nonmalignant" fall into four groups: (1) definitely healthy persons, (2) students who were apparently in good health, (3) patients admitted to hospital without organic disease as far as known (hernia, fracture, hemorrhoids), (4) various medical and surgical cases considered to be certainly nonmalignant. Some of these gave positive results. Of these, details were available in six, two being cases of suppuration, one of tuberculosis with pyopneumothorax, one of simple goiter, one of Hodgkin's disease, and one a chronic mastitis (therefore possibly precancerous).

A considerable number of "suspect" cases could not be included in the table, as it was felt that pathologic confirmation was desirable before the case was eligible, considering how effectively other diseases sometimes mimic cancer.

Patients under Radium Treatment.—A definite fall in the proportion of patients who show these bodies is noticed, and in the others a longer search was required before they could be discovered.

No note is here made of the relation between positive findings and *stage of disease.* It has been found that even histologically, early cases may show the inclusions. But some of the "negative" results were furnished by clinically early cases.

DISCUSSION

Filamentous and other cytoplasmic structures are considered by cytologists to be integral components of any cell. But they are not specially referred to in textbooks on hematology as being discernible in ordinary blood films. The exact counterparts of those under discussion are, however, figured in, for instance, E. B. Wilson's classical work on *The Cell.* Similar bodies are described and discussed in the literature on virus diseases, and the question as to their nature and significance may be discussed on the same lines.

A. They may be intrinsic in the cell: (i) normal products of cell metabolism, or of growth, or of cell division. Here belong mitochondria, filamentous chondriocontes, beaded chondriosomes, lepidosomes, metachromatic, secretory, or other granules, Golgi apparatus;[9] (ii) nuclear in origin; chromidia, spirem threads, chromosomes; (iii) degenerative products.

One has to decide between (i), (ii), and (iii) before deciding that a given cell really contains the particular bodies in question. Granules are not noted. Hence, the difficulty of deciding whether a granule is a degenerative product or a secretory product is evaded. The position of *filamentous mitochondria* in the cell economy is also not agreed upon by cytologists. For the present purpose, it is, however, convenient to refer to these bodies as mitochondrial, though they do not give the classical staining reactions.

Definite mitochondria can be seen quite frequently in neutrophile leucocytes, especially in pyogenic infections, as well as in malignant disease, presumably owing to the depletion of the granules, which renders them visible. But the similar bodies in the monocytes do not give the same staining reaction with Leishman stain.

They may be classified as chondriosomes in that the latter are regarded as phospholipoid in nature[9] (pp. 47 and 223), and Giemsa will stain such material the same way as it does these bodies.

ii. Nuclear origin: Chromidial extrusions are commonly seen in circulating blood cells, and they have been noted especially in cases of malignant disease. They are also numerous in health under a purin rich diet.[3] But these extrusions never attain the length of the bodies in question; they are of drumstick or spiculated form. Perhaps the occurrence of long ex-nuclear lines likewise denotes increased nuclear activity, material passing out into the cytoplasm.

The noded lines may be of nuclear origin also, if one regards the cytoplasm as permeated by canaliculi down which granules of nuclear matter proceed outward into the circumcellular fluid. The internodal lines would then be taken as optical effects produced by the canaliculi. The mycelioid forms might be

explained in a similar manner, the ex-nuclear material running into the canalicular network without separating into droplets or granules.

Such an explanation would justify taking the presence of these bodies in general as *an index of chromoplastic activity,* and this would be in harmony with the generally favored view as to the nature of malignant disease.

The noded lines bear a resemblance to linin threads. But this is likely to be accidental, for there is no reason why they should enter the cytoplasm *en masse,* especially considering that circulating leucocytes do not undergo mitosis. Portions of chromatin have, therefore, no opportunity to become detached or to fail to reenter daughter nuclei. In any event iron hematoxylin has not shown their presence.

B. They may be extrinsic to the cell. (i) Nonspecific to malignant disease: (a) artefacts, such as contamination with extraneous objects, pseudospirochetes, physical staining effects; (b) phagocytosed organisms. (ii) Specific to the disease (a) in etiologic relationship, (b) not so.

Re (i) (b). In favor: (1) The Staining reaction. On the one hand, ordinary pathogenic bacteria do not stain red with Leishman stain, whether free or within neutrophile leucocytes. On the other, experiments made by me have shown that protozoa and mycomycetes from insect intestine stain the same color as to these mitochondrial bodies after phagocytosis by human leucocytes, using opsonic technic. In the case of the leucocytes in malignant disease both neutrophiles and monocytes may show the bodies, yet those in the neutrophiles stain like ordinary bacteria, whereas the others take on the red color. It is, therefore, feasible that one is dealing with phagocytosis in each case, but the type of organism is different or its reaction in the cell is different. (2) Carcinomatous tissue frequently contains bacteria. Ulcerated tumors are necessarily infected. But even other tumors prove to contain various organisms. It would appear that neoplastic tissue attracts various organisms which may be circulating (as they do even in health), so that they gradually accumulate in the tissue.[8] A number of recent publications discuss the bacterial content of tumors and suggest it as a cause of increased virulence of the neoplasm, especially in the cervix cases.[1, 7] In fact, bacteria might even be necessary to the growth of the neoplasm, so that the therapeutic attack should commence upon them.

Now the flora of these infected tumors is multiple. They, therefore, may provide both types, those taken up by the neutrophiles, and those by the macrophages, with the corresponding difference in staining effect. (3) Histologic study shows that there is at least every opportunity for both orders of cells (invariably present in and among the tumor cells) to take up these organisms and return to the blood. Fragments of mycelium, and noded or geniculate bodies have been identified in specimens from cases in which the blood has shown the inclusions.

Against: (1) even though ingested by monocytes, even the higher bacteria should be stainable with special bacterial stains, yet they are not. (2) There must be enormous numbers of these organisms circulating in the cancer case, seeing that some are present in every cubic millimeter.

Re ii. (Are they specific?) (a) In etiologic relationship? In favor: (1)

They are found in the tumor cells also; (2) some authorities on virus diseases incline to the idea that the inclusions in those diseases are specific; (3) absence of ordinary staining reaction for known bacteria (at one time the mycelium of actinomycosis could not be demonstrated in the tissue-lesion); (4) the nodes in the geniculate bodies may be similar in function to the arthrospores of streptococci, etc., or of streptothrix.

Against: (1) The evidence that they may be simply phagocyted organisms which certainly do not cause cancer; (2) tumor cells are able to phagocyte such organisms (but the presence of such bodies is taken as itself evidence that tumor cells can ingest organisms); (3) most workers do not regard inclusion bodies in virus diseases as causal (on the other hand, this is merely an expression of opinion, either way); (4) they have not the individuality, e.g., of malarial plasmodia.

(b) Not in etiologic relationship? That is, are these bodies not the actual virus, but vectors of virus? In favor: (1) There is nothing intrinsically impossible in ex-nuclear material being a bearer of ferment or zymogen, seeing that intracellular ferments must inhere in some concrete cell constituent anyway. Therefore, such material can equally harbor a virus. (2) There is the analogy of the chromosomes, which bear highly complex potentialities.

Whatever be the origin and nature of these bodies, there remains one practical fact, namely, that the subject of malignant disease has his blood "loaded" with the material in question, even at an early stage in the case. Therefore, if the answer to question B ii (a) is positive, it would mean that the monocytes carry virus about the body, and that metastases might arise even if tumor cells did not become detached. The whole body would be diseased almost from the outset, and it would not be a matter simply of an initial local lesion. But if the answer to question A ii be positive, the findings in the blood films would be interpreted in the sense of a nonspecific chromoplastic activity, and one would understand why such conditions as extensive abscess formation sometimes also furnish similar bodies in the blood cells.

On that last view, the findings may simply reflect the outpouring of cytotoxic and cytotaxic products by the neoplasm itself, an event which need not be continuous, and need not occur in all stages. The new tissue may be "closed" by fibrosis, or "open" (only a lymphocyte barrier). In the former case the mononuclear leucocytes reveal nothing, whilst in the other case they contain these bodies. It has been found in the course of this study that the blood is actually not always equally rich in the mitochondrial bodies even at all times in the day. The nucleo-irritative products seem, therefore, to vary in amount, apart from the defensive tissue reaction. Perhaps this defensive reaction explains the disappearance of the inclusions after successful radium therapy.

It may be noted that in a few cases the benign or malignant nature of a palpable tumor could be decided correctly by means of this search for cell inclusions.

SUMMARY

A description is given of certain filamentous, spirillar, and geniculate structures seen in (especially) the monocytes in a high percentage of cases of malignant disease, and in very few cases which were clinically nonmalignant.

They are demonstrable only with Giemsa staining or by Leishman's stain.
They may disappear from the blood after successful radium therapy.
Their possible nature is discussed.

During the past year, liberal facilities for bringing this study to maturity were furnished by Dr. E. W. Archibald, Director of the Department of Surgery of McGill University, so that material became accessible from the Royal Victoria Hospital, the Montreal General Hospital, and other hospitals in Montreal. Thankful acknowledgment is here extended to the physicians and surgeons who placed their cases at disposal.

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THE TOXEMIAS OF PREGNANCY*

II. The Nitrogen Metabolism

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In a previous communication[1] the senior author has discussed some evidences of the hepatic factor in the group of widely diverse conditions generally called the "toxemias of pregnancy." These evidences were among the results of an elaborate chemical and laboratory study of fifty pregnant women who exhibited some one of these toxic conditions.

The basis of selection, the method of study, and the general composition of the group have already been considered in the paper noted above.

Data derived from the study of seventy-seven women studied repeatedly both throughout a normal pregnancy and for a number of weeks thereafter,[2] have served as a control; these will be drawn upon, as needed, for the present discussion.

This present paper is concerned with the protein metabolism, as evidenced chiefly in the levels of blood and urine nitrogen.

*From the Evans Memorial, Massachusetts Memorial Hospitals.
Received for publication, July 31, 1933.
Read before the Section on Pathology and Physiology at the Eighty-Fourth Annual Session of the American Medical Association, Milwaukee, June 16, 1933.

The normal group was in part drawn from institutions for unmarried mothers, who were usually young primiparas. The toxemia group were older women, only one-third of whom were in their first pregnancy and not a few of whom gave histories of extensive childbearing. No evidence was forthcoming in the study that repeated fetation led to results differing from those characterizing the primiparas.

The time of study of the individual members of the toxic group was varied. Three-fourths of the patients fell in the last three months; the remainder showed only a general distribution.

In considering the protein exchange, the total nitrogen in the urine is

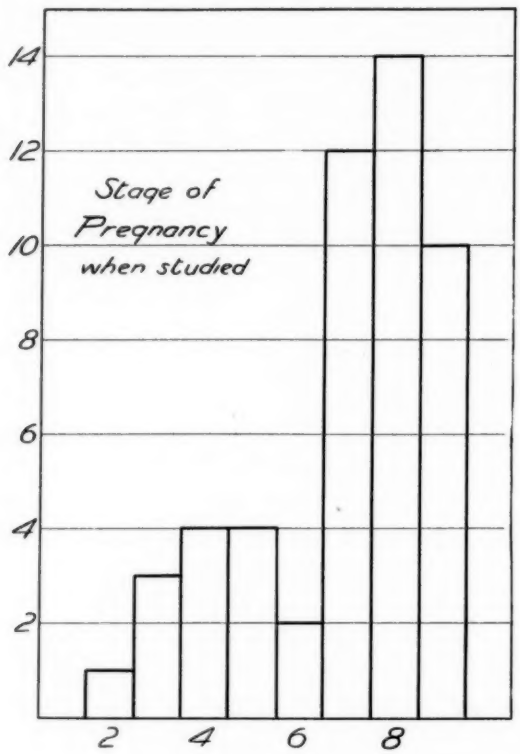

Chart 1.

significant as a measure of the absolute protein utilization, since skin elimination under most circumstances is but an inconsiderable fraction. Further, the independent quantitation of several individual components whose summation determines the total value is significant, as the metabolic origins of each fraction are widely differentiated. The original metabolized protein is the sole factor common to them all. To illustrate, urea is the indifferent form in which the principal amino-acid waste is eliminated, while ammonia, on the other hand, is the end-result of a defense mechanism mediated by the kidney to aid in the elimination of certain acid metabolites without depleting the blood of its essential fixed alkali. The so-called "nitrogen partition" deals with the four major components, urea, uric acid, ammonia, and cre-

atinine, and then assembles the remainder of the numerous nitrogen-containing substances into a single fraction called the "residual" nitrogen, which is determined by difference. Folin[3] established certain criteria for normal partition values and the few later studies on normals (Matthews,[4a] Long and Gephart,[4b] Rowe and Proctor[4c]) have been broadly confirmatory. The last authors[4c] confirmed earlier observations that the residual fraction showed a relative increase when the total nitrogen was diminished and by a synthesis from their own data, with those of Folin (l.c.) and Smith,[5] defined the curve representing this relationship.

Briefly, with a total elimination exceeding 8 gm., there is seemingly a fixed relationship at something less than 6 per cent. At levels slightly below this minimum the curve takes a sharp progressive upward trend and soon assumes large magnitudes. In a number of studies the senior author has defined the upper normal level of residual nitrogen at 9 per cent where the total elimination indicates a protein exchange above the conventional main-

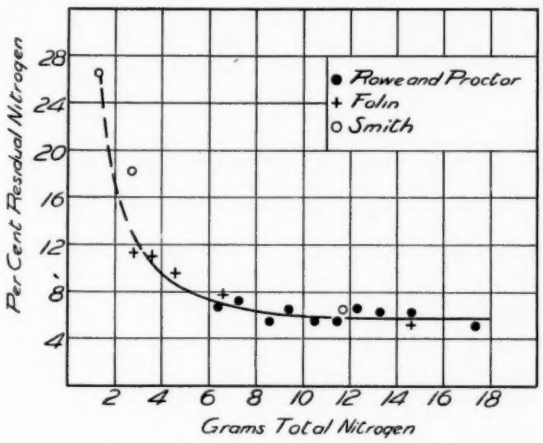

Chart 2.

tenance level of 7 gm. of N_2. Further, he has shown that values above this rather generous limit are one general index of a disturbed metabolism arising from a very wide variety of causes. This value then assumes a special significance in the assessment of the normality of the individual metabolism. In an earlier paper[6] dealing with the normal control group, the average nitrogen elimination was reported as relatively constant, at least during the last twenty-four weeks of pregnancy, and lying at a level slightly superior to maintenance. When one remembers, however, that the mother is storing protein in amounts far in excess of fetal needs, the adequacy of the catabolic exchange shown by the urine level assumes an added significance.

Turning now to the actual results of the study, the significant figures are grouped in tabular form (Table I).

The identity of the two total nitrogen values in the normal series ante- and postpartum, is fortuitous. Comment should be made, however, on the fact that following delivery the antepartum storage seems to be replaced by a different factor which also produces the common end-result of low urine

TABLE I

NITROGEN PARTITION IN URINE

DATUM		NORMAL		TOXIC	
		A.P.	P.P.	A.P.	P.P.
Total nitrogen	(gm.)	8.03	8.04	6.93	8.33
Urea nitrogen	(%)	79.6	80.8	77.8	80.8
Uric acid nitrogen	(%)	2.8	1.9	3.1	2.8
Ammonia nitrogen	(%)	5.0	5.1	4.2	3.3
Creatinine nitrogen	(%)	4.7	4.2	4.7	3.7
Residual nitrogen	(%)	7.9	8.0	10.2	9.4
Per cent with residual more than 9%		37	33	65	48

nitrogen. Naturally, the actual loss of protein through the breast milk comes into consideration here. Urea is slightly low in both periods, though postpartum it evidences a possible beginning recovery to the usual normal levels. This is no more than yet another report of the low urea which has come to be

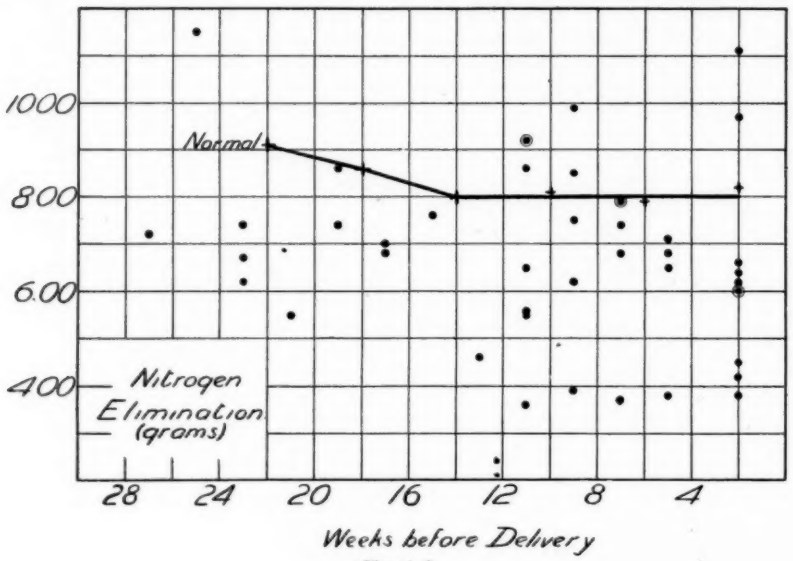

Chart 3.

recognized as a normal feature of pregnancy. A high uric acid during pregnancy shows a drop to a more normal level when the strain of gestation is removed; ammonia is slightly but definitely high; the average for residual nitrogen is well within the normal range, but about one-third of the series show values which are absolutely high.

In the toxic group, several differences at once present indicating the general disturbance of metabolism associated with the toxic status. The total nitrogen is significantly lower. Even though this could result from an impaired appetite and lowered food intake it would still be an evidence of toxicity. To render this depression more apparent, the individual measurements are plotted in Chart 3. The curve marked "normal" derives from the average data of the control series and serves as a delimiting boundary. But few of the observations fall above its level, while the great majority are significantly below.

Of the other figures, urea is still lower, uric acid still higher, while the residual fraction is frankly above the liberal allowance for the normal, and two-thirds of the group participate in this departure. With the emptying of the uterus, the nitrogen rises to a level normal in relation to the control group, urea rises relatively and absolutely, and the residual nitrogen falls both in amount and in the frequency of report of high levels. As these women were examined during the fortnight following delivery, the time factor bulks large and the trend toward normality becomes even more significant of a changed status. Ammonia is lower in the toxic than in the normal series. In the postpartum period it more closely approaches the conventional normal than does that in the control series. Loss of fixed alkali through the milk should affect both groups equally and the explanation of the anomaly is not directly forthcoming. The originally high uric acid shows a slight downward trend but remains at a height which is frankly above the normal. Probably this reflects the hepatic factor present in so many of the cases; hepatic dysfunction is known to modify purin metabolism, and these cases present a functional derangement which but slowly returns to the normal. The liver complement in the picture could also account for the high residual nitrogen figures.

To summarize this section briefly, pregnancy as such, produces changes in the urine nitrogen suggestive of some degree of toxemia, while actual toxicity during this condition accentuates these changes. Further, with the removal of the common causal factor of gestation by delivery, even in the toxic group there is evidence of a prompt retrograde movement toward normal relationships.

The various dissolved nitrogen-containing substances in the blood usually designated as the "nonprotein nitrogen" are, with but one significant exception, ammonia, the forerunners of the urine nitrogen in its many subdivisions.

In reviewing what may be designated as the "nitrogen partition" of the blood, only the ammonia is lacking from the group. Convention and propriety further dictate a different method of recording the blood constituents to express more significantly the meaning of departures from the normal. The urine is a summation of the various secreting levels of the twenty-four hours during which, by thermodynamic work, the blood is concentrated to form the urine. This collects in the bladder, a container inside the body, and is emptied from it at intervals into other containers only relatively more remote from the secreting agent. The blood, on the other hand, is the channel of ingress and egress of foodstuffs and wastes, and the levels of its several constituents are maintained at a relative constancy through the agency of the vast number of individual factors contributing to the continuance of homeostasis. Under the force of superimposed effects, new equilibria are established at other levels but the tendency always is to return to the earlier conditions when the extraneous agencies cease to operate. If the pressure be not too great, the homeostatic influences tend to compensate for such effects and restore or maintain normal levels even during the continued operation of the added effect. Temporary changes in blood sugar levels during sugar absorption

and the maintenance of blood P_H in compensated ketosis are typical illustrations. The absolute levels in the blood thus assume a significance that in the urine is largely absorbed by the single factor of the total nitrogen. The average blood levels ante- and postpartum in the two series are collected in Table II.

TABLE II

BLOOD NITROGEN

DATUM		NORMAL		TOXIC	
		A.P.	P.P.	A.P.	P.P.
Nonprotein nitrogen	(mg.)	25	32	28	32
Urea nitrogen	(mg.)	12	16	14	15+
Uric acid	(mg.)	3.3	3.7	3.6	3.6
Creatinine	(mg.)	1.5	1.5	1.5	1.5
Residual nitrogen	(mg.)	11.3	14.2	12.2	15.2

It has long been a well-recognized fact that the nonprotein nitrogen and its composing fractions are, in the main, definitely depressed during pregnancy. We have never been able to record the sharp additional decline in blood urea to levels approaching 30 per cent of the whole, reported by several observers; we have uniformly found the values in pregnancy to coincide with those in normal males and nonpregnant females, or from 45 per cent to 50 per cent. In the present instance both series show an entirely normal relationship. On the other hand, uric acid values show a modest upward tendency, although in normal pregnancy individual values may fall within the conventional normal limits. In relation to the nonprotein nitrogen, however, the uric acid values are definitely high. It is apparent that antepartum all of the values, barring creatinine and uric acid, are definitely low, and that delivery engenders a prompt return to normal levels. In the toxic series, the values antepartum are all somewhat higher, a presumptive evidence of low-grade retention that is in harmony with the recognized renal influence in this condition. After delivery the blood reverts to normal levels that are very close to those of the normal controls. Recognizing that the homeostatic agencies operate most effectively in the blood stream and that existing chemical methods are incompetent to record very small variations, the blood levels fail to give evidence of that slower return to normal levels that is clearly shown by the urine data from the toxic group.

To summarize briefly, pregnancy causes a lowering of the nitrogenous constituents of the blood, uric acid excepted, which is less marked in cases of toxemia as the renal element here introduces an opposing influence. Both normal and toxic pregnancies tend to revert to the usual blood levels shortly after delivery.

CONCLUSION

1. The blood and urine data reflecting the protein metabolism have been determined in series of normal and toxic pregnant women.

2. Characteristic urine findings are recorded with pregnant patients; the hepatic and renal features of toxemia are superimposed influences adding their special effects to those of the basic condition.

3. Termination of pregnancy initiates a return to normal levels, the latter less rapid in the toxic group.

4. Blood nitrogen levels are low in normal pregnancy. Toxemia tends to develop the renal influence as an opposing agency.

5. Restoration to normal blood levels follows rapidly on the termination of the pregnancy in both study groups.

The authors express their indebtedness to all who have participated in the study.

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VERRUCOUS AORTITIS WITH SPECIAL REGARD TO ANEURYSM FORMATION IN CHILDREN*

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FOR a long time, nonsuppurative inflammatory lesions in the aorta were attributed chiefly to syphilis, a conception first expressed by Doehle in 1885 and later in 1899 by Heller.[1] The "Doehle-Hellersche Aortitis" held sway over medical thought until more recent investigations revealed that acute infectious diseases as typhoid fever, typhus, influenza, and scarlet fever, may also cause inflammatory changes in the aorta (Siegmund[2]). Of particular importance, however, is the fact that rheumatic fever seems quite frequently to leave its mark on the large arteries (Klotz,[15] Pappenheimer and von Glahn[16]).

Although inflammatory changes in the aorta were suspected in the course of rheumatic fever, no sustained effort to define the histologic features was made until the work of Klotz[15] in 1912. Clinically, the diagnosis has been made many times, but it is unverified by autopsy.

To this category of unverified diagnoses belong the reports of Bouillaud[3] (1840), Roger[4] (1863), LeLong[5] (1869), Lauenstein[6] (1876), Leger[7] (1877), Marfan[8] (1901), Méry et Guillemont[9] (1902), La Rue[10] (1903), Renon[11] (1905), Weil et Menard[12] (1912). Bernert[13] (1910) deserves the credit for calling at-

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Received for publication, August 12, 1933.

tention to the fact that many of the previously reported cases, cited as rheumatic in origin, were probably examples of mycotic aneurysms. Rabé[14] (1912) was the first to present histologic findings.

In 1912 the first serious effort was made by Oskar Klotz[15] to study the histologic lesions in the aorta occurring as a result of rheumatic infection. In 15 cases of rheumatism which came to necropsy, he had 3 patients, aged nine, sixteen, and twenty years, dying during the first attack of rheumatic fever; 4 cases of patients, aged twenty-eight, thirty, thirty-six and forty-four, with history of recurrent attacks; and 8 cases of patients with chronic rheumatism, all of whom had suffered from recurrent or progressive heart disease associated with rheumatic fever. He concludes that there appears to be a fairly definite form of arterial disease which is associated with rheumatic fever in its different stages. The arteries react to the irritant in a true inflammation, and this reaction is observed in the adventitia and the outer portion of the media. In the acute stages, the inflammatory exudate is of the nonsuppurative variety in which the lymphoid cell is most prominent. The inflammation occurs particularly in the neighborhood of the smaller arteries, while the medium-sized vessels are little affected. On the other hand, the larger arteries, which are supplied in their outer coats by nutrient vessels, are damaged by the inflammatory process, which travels along the vasa vasorum. He observed, that during the acute process, the changes in the aorta consist of a destruction of tissue noted in the loss of muscle and elastic elements. Similar degenerations are common in the heart. At times the degenerative processes are extensive, leading to the loss of considerable tissue in, and probably weakening of, the media. The intima of the arteries, he states, does not appear to be primarily affected. In the early stages, slight superficial fatty changes are sometimes noted, while later in the chronic stages the intima proliferates, giving rise to a nodular endarteritis.

In 76 cases of acute rheumatic fever, Pappenheimer and von Glahn[16] found the distinctive lesion of the aorta to be a scar, roughly the shape of an inverted wedge, often acellular and located in the region of the nutrient vessels of the media. This type of arterial lesion was not found in a comparative study of 77 nonrheumatic cases, with the exception of one, in which death resulted from pneumococcic meningitis. In the aortic adventitia, they found specific changes consisting of the presence, in some cases, of typical Aschoff cells, showing, however, little tendency for grouping. These cells, they regard as diagnostic. They are rounded or oval. The cytoplasm is basophilic and stains intensely with pyronin. The nuclei each have a distinct nucleolus, and the cells seem to be arranged about degenerating collagen fibers. In their second communication on this subject, the authors described earlier reactions in the media to support the view that the perivascular medial scars are the sequellae of an earlier focal perivascular inflammatory lesion.

Following the first paper by Pappenheimer and von Glahn, many communications began to appear, both in American and European literature, giving histologic evidence of destructive effects of rheumatic fever on the aorta (Weisl,[29] Besançon and Weil[30]), even so severe as to lead to aneurysm formation (Gray and Aitken[31]).

Changes in the adventitia of the aorta were further emphasized as perivascular round cell accumulations and nodular fibrosis (Chiari,[18] Barnard[20]). Clawson[21] injected *Streptococci viridans* intravenously into monkeys and produced changes in the media and adventitia similar to those seen in rheumatic fever. He also gave subcutaneous administrations of the cocci to rabbits but produced only subcutaneous nodules similar to those described by Swift[22] and Frank.[23]

Sacks,[28] in his review of the pathology of rheumatic fever, states that since the vasa of the pulmonary artery are derived from the coronary arteries, lesions in this artery are to be expected. Kugel and Epstein,[24] on the basis of this supposition, examined a series of 24 cases of rheumatic fever and found 5 in which there were histologic evidences of active inflammatory changes in the pulmonary artery. The changes in the pulmonary artery were of two types; namely, a diffuse cellular infiltration in the intima and subintimal layers of the media and focal perivascular accumulation of cells comparable to the Aschoff nodule in the myocardium. In 2 cases, there were macroscopic changes in the intima consisting of small, raised, dull gray yellow patches of irregular outline. In 2, there was a disruption of the elastic fibers of the media, almost as conspicuous as in syphilis. In 14 cases, the pulmonary valve was involved showing an interstitial valvulitis. In 3 cases, Aschoff bodies and in 6 verrucae were found. In 17 cases, in which there were changes in the musculoarterial junction, Aschoff bodies were found in a few. The aorta was involved in 22 cases. In 4 cases, gross lesions in the intima were present. As controls, they used 31 cases in which there was a nonrheumatic pericarditis, and in 75 cases no pericarditis or rheumatic cardiac disease was present. They found nothing in any of the controls which was in any way confusing.

Destructive lesions in the media with extensive scarring were also reported by Paul,[27] in the course of rheumatic fever, and Wätjan[26] observed Aschoff bodies in the epicardial fat over the anterior surface of the pulmonary artery. Gotthardt[25] reported a case of verrucous endocarditis of all the valves and fibrinous pericarditis which were accompanied by an endarteritis of the pulmonary artery. Chiari[19] found a fibrosis of the adventitia of this artery.

PRESENTATION OF CASES

In a series of 4,100 consecutive autopsies, 3 cases have been found in which there was a verrucous lesion on the intima of the aorta.

CASE 1.—An Italian female child, eleven years old, entered the Cook County Hospital on March 8, 1932. She was apparently well until two weeks prior to entrance when she developed pain over the heart, cough, and fever. On the day of entrance into the hospital, she had a moderate nose bleed.

The birth and development history was negative.

The child had had measles and chickenpox.

On physical examination, the patient was found to be well nourished but somewhat undersized. The temperature was 102.4° F.; pulse, 124; respiration, 28; and blood pressure, 140/60. The skin was dark and there was long, dark hair over the upper lip and along the side of the cheek, on the back and a little over the lower extremities. The pubic hair was luxurious and of female distribution. There were diffuse coarse râles heard over both lung fields.

The heart was enlarged transversely and there was a soft, systolic thrill and a systolic and diastolic murmur over the apex. Over the aortic area, indistinct murmurs were heard.

The pulse at the wrist was forceful, of high pulse pressure, quickly striking the finger and receding. The carotid pulsations were easily visible. The femoral, popliteal, dorsalis pedis, and posterior tibial arteries were not palpable, nor could they be detected by the blood pressure apparatus.

The liver was palpable, but the spleen was not.

The white blood count was 20,100 with 84 per cent polymorphonuclear leucocytes. There were many macrocytes present. Polychromatophilia and anisocytosis were evident. The urine was negative as was also the blood Wassermann.

The clinical diagnosis was endocarditis of the aortic and mitral valves, diffuse bronchopneumonia, and coarctation of the aorta.

While in the ward, the patient was rather comfortable but ran a low grade remittent fever of 99° to 100° F. The findings in her lungs spread gradually. About one week after entering the ward, the child was seen sitting up in bed and resting quietly but coughing frequently. Forty-five minutes later, she was found on the floor dead.

Autopsy.—(Performed by Dr. R. H. Jaffé.) The essential findings were in the heart, aorta, and incidental interesting findings in the genitalia.

The heart weighed 250 gm. The wall of the left ventricle measured 12 mm., and that of the right ventricle 5 mm. in thickness. The epicardium was thin, transparent, smooth, and shiny. The apex was formed by the left ventricle. The myocardium was purple, gray and friable. The transverse and vertical diameters of the left ventricle were 8.5 cm. The trabeculae and papillary muscles were slightly flattened. On the auricular surface of both cusps of the mitral valve, were present pinhead-sized, granular, firm, and slightly transparent nodules. The free edges of the aortic leaflets were thickened and rounded, and along the limbus there were firm, sandlike, granular deposits present.

The aorta measured 52 mm. in circumference above the valve. About 1 cm. above the aortic valve, there was an oblique, transverse tear which was 35 mm. wide. The edges of the tear were indented, and 2 mm. longitudinal bridge of intact wall separated the tear into a larger right and smaller left portion. The floor of the tear exposed a thin, pink gray membrane with fine, longitudinal lines and small, granular elevations. The adventitia was injected. To the right, the tear terminated in an irregular, slightly depressed, light brown area. In the ascending aorta, there were several up to 25 × 4 mm. streaklike, light yellow areas. Just beyond the origin of the left subclavian artery, the lumen of the aorta becomes suddenly narrowed to a diameter of about 1 mm. Beyond the narrowed portion, the circumference of the aorta widened to about 58 mm. Small, light yellow plaques were found in the upper wall of the arch. At the diaphragm, the circumference of the aorta was 36 mm., and below the origin of the renal arteries it was 22 mm.

Above the bifurcation of the aorta, there was a worm-shaped, loose, cylindrical, light pink gray blood clot 55 mm. long and 10 mm. in transverse diameter. The lower end of the blood clot projected into a flagellum-like extension 35 mm. long. The upper end terminated into a pointed projection 3 mm. long. The circumference of the aorta above the bifurcation was 18 mm. The internal mammary artery had a circumference of 6 mm.

The uterus measured 45 by 25 by 8 mm.

External genitalia: The clitoris was about twice the normal size. The vestibulum vaginae and vagina were well developed. The vulva appeared rather narrow because of an enlargement of the labia minora.

Ovaries: The right ovary was replaced by an ovoid body, 18 by 10 by 6 mm. in diameter, which showed a distinct, light pink yellow capsule, and on sectioning, was found to be composed of a finely granular, light yellow tissue. The left ovary was missing, and in its place was found a ridge 25 mm. wide and 3 mm. high.

Microscopic examination:

Aorta: The tear in the ascending portion of the aorta involved the intima and the entire media and exposed the adventitia which was densely infiltrated by polymorphonuclear leucocytes. Approaching the tear, the intima became slightly thickened, and in the region of the tear, was everted, covering the edges of the torn structures. The thickening of the intima resulted from a cellular accumulation between endothelium and internal elastic membrane. Delicate elastic fibrils were separated by swollen fibrocytes with large, pale nuclei, small round cells, and histocytic elements with an ample cytoplasm and round or oval nuclei. There were bizarre-shaped, drawn out nuclei free between the intact cells. The internal elastic membrane was split into several layers and in the everted portion showed areas frayed to the extent that it was completely interrupted. Near the torn portion, the muscle and elastic fibers of the media passed rather abruptly into a zone of complete necrosis without structural differentiation and scanty chromatin granules. The necrotic area stained from brass yellow to deep yellow brown after van Gieson and pink red with eosin. In the outer portion of the media, single mononuclear elements were wedged in between trabeculae of necrotic tissue,

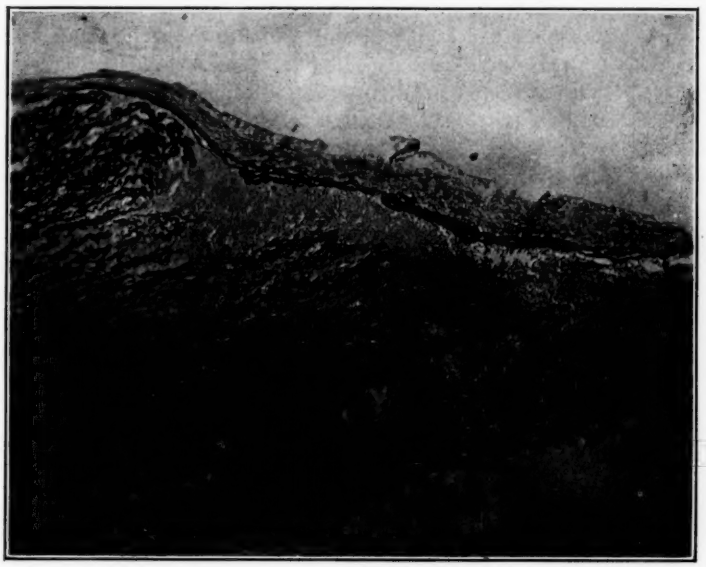

Fig. 1.—(Case 1.) Aorta. Section taken through the area of rupture. Elastica stain (×100).

and the necrosis extended for a short distance beyond the tear along the external elastic membrane. In the floor of the defect, there were only a few small segments of the external elastic membrane visible.

Between the polymorphonuclear leucocytes which infiltrated the adventitia, were found single, large, degenerating fibrocytes, the nuclei of which could assume very irregular forms. Elastic fibrils were practically absent.

Away from the tear, the intima appeared well preserved. In the media, there were circumscribed areas of increase of the metachromatic substance which spread apart the elastic elements. Near the outer elastic membrane, there were small areas in which the coarse elastic fibers seemed to have disappeared while the fine fibrils still could be recognized. The adventitia was of distinctly increased cellularity. The fibrocytes were swollen, often separated and mobilized. There were many mononuclear cells with a distinct rim of cytoplasm, and lymphocytes and a few oxyphilic leucocytes. In addition to these cell forms, elongated cells were found with pyknotic, crenated and elongated nuclei, which could be seen also between the muscle fibers of the adjacent portion of the media.

Myocardium: There were in the myocardium, numerous nodular accumulations of large cells which were located near the larger blood vessels. The cells which formed the nodules

had a pale stained cytoplasm and oval nuclei with a central indented spindle of chromatin. These cells centered about areas of fibrinoid necrosis. Between them, a few lymphocytes and leucocytes were found. Underneath the endocardium, areas of necrosis were found, which were surrounded by cells of histocyte type distinctly different from the cells of myocyte type. In addition to the cellular nodules, there were flame-shaped scars radiating between the muscle fibers, which were increased in thickness and the striations of which were obscured.

The anatomic diagnosis was coarctation of the aorta with thrombosis of the aorta distal to the coarctation; spontaneous rupture of the aorta above the aortic valve and rheumatic aortitis with necrosis of the media; verrucous endocarditis of the mitral and aortic valve and slight fibroplastic deformity of the aortic valve; slight excentric hypertrophy of the heart; moderate dilatation of the internal mammary and intercostal arteries; chronic passive congestion of the lungs, liver, spleen and kidneys; bronchopneumonia of the right lower pulmonary lobe; bilateral hydrothorax; follicular hyperplasia of the spleen; pseudohermaphroditismus internus masculinus.

CASE 2.—A colored female, twenty-seven years old, entered the Cook County Hospital on April 23, 1932, stating that she had been well until one month ago. At this time, she began having periodic attacks in which her heart appeared to "jump." These attacks were followed by precordial boring pain that radiated to her left shoulder down to her left arm and seemed to stay there. The attacks were easily precipitated by any exertion or excitement. At the onset the attacks lasted but a few minutes, but recently had increased in length up to twenty minutes or more. Often during the attack, the patient fainted, but usually she had to stop immediately in her work. There was no great fear of death at any time. Occasionally nausea, vomiting, and urinary urgency followed an attack. The patient was married twice and received three injections of mercury in her buttocks in 1922.

The physical examination revealed a well-nourished colored female, lying quietly in bed and not acutely ill. The temperature was 98.8° F.; pulse, 80; respiration, 20; and the blood pressure, 106/64. The pupils reacted to light and accommodation. The heart was not enlarged; there were no murmurs or thrills. The rhythm was regular. The chest and abdomen were essentially negative. The red blood count was 3,260,000; white blood count, 7,800; and the hemoglobin 55 per cent.

The patient had several attacks during her short stay in the hospital, some slightly alleviated by amyl nitrate, others by quinidine. On the morning of the day of her death, the patient had a typical attack, which was as follows: She was sitting up quietly in bed when she suddenly toppled from her back rest, became livid, cyanotic, stopped breathing, and then broke out into a cold sweat. The pulse was imperceptible, even by auscultation. Artificial respiration was administered for five minutes and adrenalin and caffeine injected. The patient rallied but four hours later died during a similar attack. Blood for a Kahn test could not be taken during life because the prick of the needle initiated an attack. The postmortem blood Kahn reaction was 2+ positive.

Autopsy.—(Performed by Dr. R. H. Jaffé.) The essential pathologic findings were in the heart and in the aorta.

The circumference of the aorta was 56 mm. at the valve, 30 mm. at the diaphragm, and 26 mm. at the bifurcation. In the ascending part of the aorta, above the valve, there were light yellow, slightly raised patches. Above the commissures, were gray white, 10 mm.—areas covered by fine, sandlike, granular, light gray precipitates. These precipitates extended above the right leaflet for a distance of 20 mm. In the region of the arch, there were a few whitish areas and also a slightly raised, 10 mm., pale yellow gray plaque covered by fine granular precipitate. Throughout the descendens portion of the aorta, there were many small, light yellow plaques.

The heart weighed 220 gm. The left ventricle measured 15 mm. and the right ventricle 5 mm. in thickness. The epicardium was smooth and studded with pinpoint-sized, light pink gray patches. The myocardium was light purple pink and friable. The ventricles were dilated and the trabeculae were flattened.

Microscopic:

Heart: In the inner third of the myocardium, there were irregular areas of severe alteration of the muscle fibers. The fibers had lost all structural differentiation and appeared as homogeneous bands. The nuclei were broken up into chromatin granules or were entirely missing. Between these muscle fibers could be seen a moderate number of polymorphonuclear leucocytes, and around the adjacent larger vessels were larger accumulations of these cells. Throughout the wall, many muscle fibers contained fine lipoid granules. In the epicardium, there were a few small, round cell accumulations.

Aorta: Supravalvular verrucous area. The intima of the aorta was covered by a layer of fibrinoid material with elongated, drawn out and segmented, deeply stained nuclei. Underneath this layer, the intima was thickened and cellular. Here many fibrocytes, young, smooth muscle fibers, small round cells, and neutrophilic and oxyphilic leucocytes were found. In the center of the area, the changes terminated in a large focus of necrosis which involved not

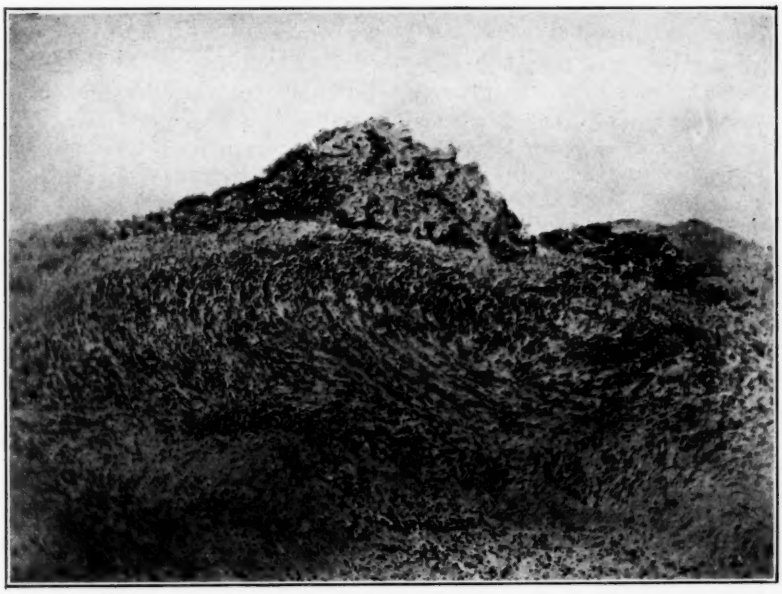

Fig. 2.—(Case 2.) Aorta. Fully developed verruca. Hematoxylin-eosin stain (×150).

only the thickened intima, but extended also deep into the media. On the inner surface, the necrotic tissue fused with the fibrinoid layer, the point of fusion being marked by streams of degenerated pus cells with karyorrhectic nuclei. Deeper down, the necrotic material showed early calcification. In the periphery of the necrosis, were found focal accumulations of pus cells; these continued beyond the necrosis between the muscle fibers of the media which here, too, were severely degenerated and partially necrotic, while the elastic fibers were swollen up and disintegrated.

Underneath the necrosis, at the border between the media and adventitia, there was a lipoid granuloma composed of pseudoxanthomatous cells, plasma cells, and small round cells, and of a few oxyphilic leucocytes. From this granuloma, tufts of cells extended into the media. In addition to the large area of necrosis, there were several smaller ones which were located in the deeper portions of the intima and in the media. They showed fine calcium deposits and were not separated from the surrounding tissue. Underneath these areas, there were perivascular accumulations of round cells, plasma cells, and eosinophilic leucocytes. The vasa vasorum had greatly thickened walls, and there were many dense accumulations of lymphocytes and plasma cells in the adventitia.

Plaque in the Arch of the Aorta: The inner surface of the plaque was covered by a thin layer of fibrinoid material which took the Weigert stain. Underneath this layer the intima was thickened and cellular. The fibrocytes were swollen and increased in number, and there were loosely scattered neutrophilic and oxyphilic leucocytes, small and large lymphoid round cells, and focal accumulations of plasma cells. In the center of the plaque, a large area of necrosis was present, which extended into the media without a sharp line of demarcation and contained fine calcium granules in the basal parts. From the fibrinoid surface layer, the necrotic tissue was separated by a strip of living tissue upon which followed a collection of disintegrating polymorphonuclear leucocytes with broken up and pyknotic nuclei. Bacteria could not be demonstrated in this area or in the necrotic tissue itself. In the region of the necrosis, the internal elastic membrane was completely destroyed, the destruction being preceded by a splitting into the finest fibrils. Throughout the media, there were many newly formed capillaries extending close to the intima and surrounded by lymphocytes and plasma cells. The piercing of the media by these vessels and infiltration was associated with a disappearance of the elastic fibers. The adventitia was thickened and fibrosed, and the arteries of the adventitia showed a very marked proliferation of the intima with much narrowing of the lumen. There were many focal accumulations of plasma cells and lymphocytes.

Midportion of Aorta Ascendens: There was a thickening of the intima with intra- and extracellular lipoid deposits in the deeper portions. The internal elastic membrane was split and accumulations of fat-filled cells were present between the separated elastic structures. The capillaries, beyond the outer third of the media, were proliferated and surrounded by a slight round cell infiltration. There was a very pronounced fibrosis of the adventitia with a thickening of the walls of the vasa vasorum, and large accumulations of lymphocytes and plasma cells were present in the adventitia.

Levaditi's stain revealed many argentaffin granules but no spirochetes in the necrotic tissue.

The anatomic diagnosis was verrucous aortitis; syphilitic aortitis; acute myocarditis; passive congestion of the spleen, liver, and kidneys; adhesions about the liver, spleen, and both ovaries.

Case 3.—A four-year-old white female child was brought into the Cook County Hospital on July 21, 1932. The mother stated that the child was well until two weeks before entrance when it developed colicky pain in the abdomen. It was also noticed that during the past two weeks the child had had a fever in the latter part of the afternoon. Development history was normal. The mother had had no miscarriages.

The physical examination revealed a well-developed child. The temperature was 103.4° F.; pulse, 140; respiration, 26. The heart was enlarged to the left. The apex beat was displaced slightly to the right. Upon examination of the chest, the breath sounds were found to be diminished in the left midaxillary line with impairment of resonance over the left apex anteriorly. The white blood count was 29,400 with 78 per cent polymorphonuclear leucocytes. The x-ray revealed a very marked cardiac enlargement.

The clinical diagnosis was pericarditis with effusion. Five days after entrance into the hospital, a pericardial paracentesis yielded 35 c.c. of a straw-colored fluid. About ten minutes later, the child became cyanotic and expired.

Autopsy.—(Dr. R. H. Jaffé.) The pericardial sac extended from the right mammillary line to the left anterior axillary line. The sac contained 250 c.c. of coagulated blood. The heart measured 70 mm. in vertical and 50 mm. in transverse diameters. The surface was covered by loosely adherent, granular light pink gray membranes. The wall of the left ventricle was 11 mm. and that of the right ventricle, 5 mm. in thickness. The myocardium was light purple pink and friable. The heart valves were grossly unchanged.

Aorta: At the valve, the aorta measured 42 mm.; at the diaphragm, 28 mm.; and at the bifurcation, 18 mm. in circumference. The intima above the valve was smooth. Beyond the arch of the aorta, 8 mm. below the opening of the left subclavian artery, located in the anterior wall, there was an oval opening 7 by 5 mm. in diameter. The edges of the opening were smooth and slightly everted. The intima was smooth except for fine, pinpoint-sized granular and slightly transparent deposits near the lower edge. The opening led into a sac

which was 7 cm. in vertical, 5 cm. in transverse, and 5 cm. in anteroposterior diameters. The sac, which was adherent to the median aspect of the left upper pulmonary lobe, bulged into the pericardial sac stretching its reflexed portion. The sac compressed slightly the left branch of the pulmonary artery. At the greatest convexity of the intrapericardial portion of the sac, there was an irregular hole 25 by 20 mm. in diameter, with indented edges. In the sac, which was lined by a whitish finely granular membrane, there was a small amount of loosely coagulated blood.

Microscopic examination:

Aorta: When the opening in the descending aorta was approached, the first change visible was the appearance of a layer of fibrinoid material which rested upon the internal elastic membrane. This fibrinoid material contained irregular, deeply stained, and shrunken nuclei. Near the opening, the layer was raised to nodular elevations which stained brass yellow after the van Gieson method. Here and there close to the internal elastic membrane, a group of better preserved oval nuclei was found forming small palisades with the nuclei

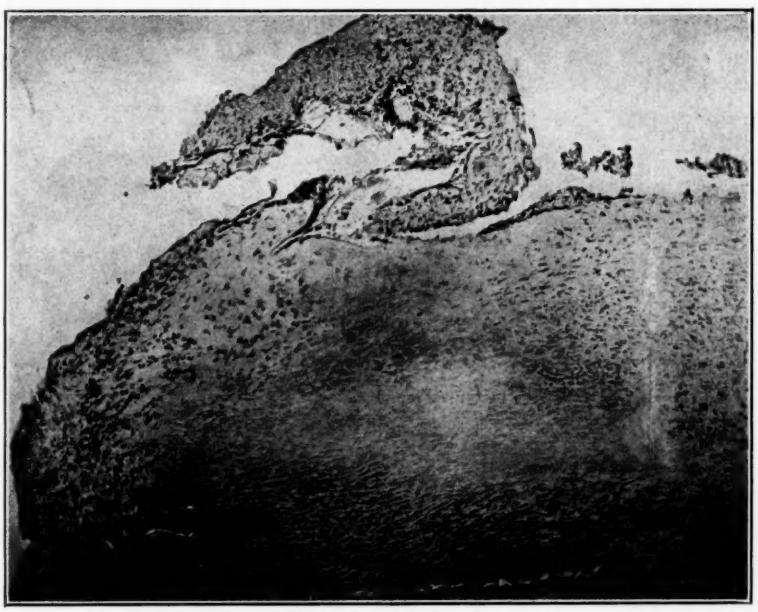

Fig. 3.—(Case 3.) Aorta at point of rupture. Hematoxylin-eosin stain (×150).

perpendicular to the membrane. At the opening, the layer of fibrinoid necrosis bent outward to pass into the internal lining of the sac. The point of bending was marked by a greater cellularity, and besides cells with shrunken and indented nuclei, there were some lymphocytes and an occasional multinucleated giant cell. Underneath the nodular elevations, the media revealed very severe changes. The muscle fibers were necrotic, hyalinized, and the chromatin granules of the broken down nuclei formed fine, dustlike precipitates. At the border of the media and adventitia, the zone of necrosis extended for some distance beyond the changes of the intima in the form of a tonguelike projection. Stains for elastin demonstrated that the internal elastic membrane was destroyed underneath the nodular formations of fibrinoid material. In the necrotic area of the media, the coarser elastic fibers were still visible, but they were broken up into small pieces and curled, and the finer fibrils had disappeared. The adventitia was well preserved and contained only an occasional small accumulation of round cells.

The wall of the aneurysm was in continuation with the wall of the aorta, the layers of which could be recognized though they were severely changed. Innermost, there was a broad band of fibrinoid necrosis with shrunken, pyknotic, and broken up nuclei, which band cor-

responded to the intima and media. Immediately adjacent to the lumen, remnants of intact tissue, which caused the internal lining of the sac to be smooth could be detected. These remnants consisted of a few connective tissue trabecles with preserved fibrocyte nuclei. The connective tissue soon passed into the fibrinoid necrotic material which was particularly rich in nuclear débris in the places corresponding to the media. In these places, remnants of elastic tissue in the form of tortuous segments were found still suggesting the arrangement of the elastic tissue of the media. Near the adventitia, mononuclear cells, neutrophilic, and a few oxyphilic leucocytes were seen between the shrunken and disintegrated nuclei of the preexistent structures.

The adventitia was the only layer which had remained intact. It was very thick and was formed by dense connective tissue interspersed by a moderate number of elastic fibrils. There were many dilated capillary blood vessels present, which were often surrounded by

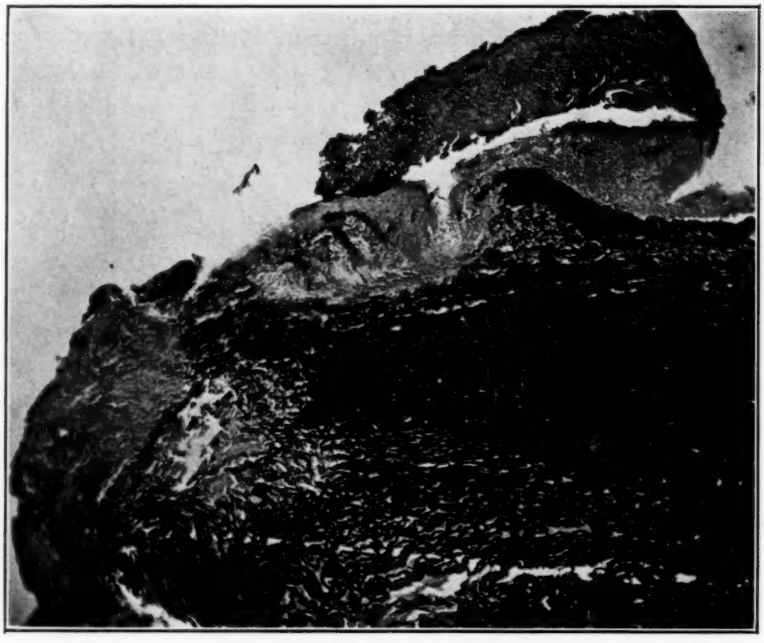

Fig. 4.—(Case 3.) Aorta at point of rupture. Elastica stain (×150).

accumulations of lymphocytes, plasma cells, histocytes, and a few leucocytes. Gram-Weigert stain demonstrated single gram-positive diplococci and streptococci in the nodular elevations on the inside of the aorta. These cocci became abundant in the region of leucocytic-histocytic infiltrations of the aneurysmal sac where they were found also inside of mononuclear and polymorphonuclear elements.

Sections taken from other portions of the aorta did not show any abnormal changes:

Myocardium: About the blood vessels of the myocardium were small scars which radiate between the muscle fibers and contain an occasional small accumulation of myocytes.

The anatomic diagnosis was verrucous streptococcic aortitis of the descending portion with formation of an aneurysm; perforation of the aneurysm into the pericardial sac and tamponade of the pericardial sac by the hemorrhage; compression atelectasis of the left lung and recent hemorrhagic infarct in the apex of the left lung; loose, fibrous adhesions about the left lung; compensatory emphysema of the right lung; cloudy swelling and anemia of the liver; hyperplasia of the lymph follicles of the spleen; slight congestion of the kidneys; ascites and left hydrothorax.

DISCUSSION

The changes in the adventitia and media of the aorta, in the course of rheumatic infection, have been much emphasized to the neglect of the important changes that may involve the intima. Few references to the occurrence of verrucous lesions in the intima of the aorta and large arteries are present in the literature. Heydloff[32] described verrucae in the innominate and carotid arteries. In one case, that of Barbacci,[33] these lesions begin 1 cm. above the sinus of Valsalva and histologically were not unlike the organizing verrucae of rheumatic endocarditis. Bacteria could not be demonstrated in them. The heart was normal.

It is quite possible that some of the cases were rheumatic in origin though no reference is made to this possibility by any of the authors. Neither are the histologic descriptions sufficiently detailed to justify any definite conclusions. It must be remembered, however, that these papers antedated the recognition of the specificity of the lesions in rheumatic cardiac disease. While many of the older writers, particularly the French authors, have ascribed to the "rheumatic virus" an important place in the causation of arterial disease, the connection was based upon clinical inference rather than histologic specificity.

Pappenheimer and von Glahn[16] recognized the possibility of invasion of the aorta by way of the lumen, as did also Perla and Deutch[34] and Kugel and Epstein.[24] Its importance, however, as the primary port of entrance has not been sufficiently stressed. Where mention of this possibility is made, it is considered unlikely for want of sufficient evidence.

In a consideration of the various types of inflammatory lesions described in the aorta in rheumatic fever, three groups may be distinguished. First, invasion by means of the vasa vasorum with involvement of the adventitia alone; more extensive inflammatory reaction may lead to involvement of the media or even to the intima and thus severe damage to all three layers. Should the acute reaction, described as the forerunner of the flame-shaped scars (Pappenheimer and von Glahn[16]), be a little more extensive or should several of these lesions be localized in one area, the resulting healing process may terminate in a gross scar; or should the reaction take place within a wall of the vasa vasorum with a subsequent thrombosis of that vessel, gross scarring may result. The rarity of these gross lesions in the aorta may be attributed either to the relatively slight involvement of the aorta in rheumatic fever, or to the belief that because the scars in the media resemble a process produced by syphilis, it is a syphilitic process. Aside from the history, the clinical picture, the serologic test, and the observations in other parts of the body, there are definite histologic differences between the scars of the media in syphilis and the scars found in cases in which they are believed to be due to rheumatic fever (Gray and Aitken[31]). Should the resultant scars or the degenerative and inflammatory process preceding these scars weaken the media to such an extent as to be unable to support the burden of the passing blood stream, a break results with a subsequent stretching of the remaining portions of the wall of the aorta, and an aneurysm forms.

Second, we may have invasion of the aorta by direct extension from an adjacent lesion (Pappenheimer and von Glahn[17]).

Third, we may have invasion from the lumen with the primary port of entrance in the intima with the formation of verrucae.

In the three cases presented here, we found one type of lesion common to all, namely verrucae. In Case 3 streptococci were demonstrated. In one case an aneurysm was present and in another, a rupture of the aorta. In attempting to link up the findings in these cases, we are struck with the common feature of all. The histologic picture of the changes in the intima are very similar to the changes observed in the formation of the nodules throughout the body in the course of rheumatic fever. Klinge[35] looks upon the widespread connective tissue damages as of close morphologic relationship. According to him, the first and essentially evident change consists, not in the cellular nodules, which are already developed, but as a degenerative change of the ground substance of the connective tissue. In a circumscribed area at the point of attack of the infecting agent, a fibrinoid swelling of the intima takes place; this is the first reaction. This stage, which Klinge[35] calls the early rheumatic infiltration (rheumatisches Frühinfiltrat), is found in the first fourteen days of the affection. It is characterized by an edema of the connective tissue and in the single bundles of fibrils; wax-like refractive masses are seen with the fibrils failing to stain with ordinary methods. When the damage is very severe, necrosis results.

In the light of these findings, we can interpret the formation of the verrucae as follows: first, there is a fibrinoid swelling, in a circumscribed area of the intima, at the point of attack of the infecting agent. The fibrinoid swelling progresses to such an extent that it rises above the surface of the unaffected intima. Later, it is followed by a proliferation of the cells of the intima so that a definite firm nodule results. The proliferating cells are chiefly of fibrocyte type with a varying amount of small round cells. The affection may either subside leaving in its wake a plaque, or it may progress, when regressive changes in the form of necrosis become more prominent. The inflammatory and necrotic changes may advance into the deeper layers breaking through the internal elastic membrane and involving also the media. The destruction in the media may be so extensive as to cause a break in the continuity of the wall of the vessel under the force of the blood pressure. Thus verrucous aortitis may lead to a spontaneous rupture of the aorta with subsequent death from hemorrhage. It may also lead to the formation of an aneurysm due to a break in the continuity of the wall of the vessel sufficiently limited to allow the other parts of the wall to become stretched and transformed into a sac. This sac may later rupture, as for instance in our case; in which the aneurysm broke into the pericardial sac. Invasion by granulation tissue from the outside may also lead to the formation of an aneurysm by disrupting and weakening the muscle and elastic fibers of the media, as was previously pointed out.

These verrucous lesions should not be confused with thrombi since they are the result of productive and regressive changes of the intima. There is no thrombus formation primarily. It is not a thromboendarteritis. In their morphology, the vascular verrucae are very similar to, if not identical with, the valvular verrucae.

The question will arise as to the specificity of the verrucous aortic lesions. In Case 1, the myocardium revealed nodular accumulations of large cells located

near the larger blood vessels. They presented the characteristic picture of Aschoff nodules. In the first case, therefore, we can definitely state that the verrucous aortitis is on a rheumatic basis. In Case 2, there were severe alterations of some of the muscle fibers of the myocardium. Between some of the muscle fibers and around the adjacent larger vessels, there were accumulations of polymorphonuclear leucocytes. Thus there was present in this case an acute myocarditis, but not specific of rheumatic fever. In this case also, there was a 2+ positive Kahn reaction on the postmortem blood, and histologically there was found evidence of a typical focal syphilitic mesaortitis. The syphilitic aortitis, however, showed no topographic or morphologic relationship to the verrucous lesion on the intima of the aorta. In the third case, there were present small scars about the blood vessels of the myocardium, which contained an occasional small accumulation of myocytes. These scars may, perhaps, have been the result of rheumatic fever, but nothing was found to substantiate this diagnosis. In Case 3 also, cocci were demonstrated. Whether these cocci have any definite relation to rheumatic fever, we are not prepared here to say. But we can, however, state that streptococci may cause lesions very similar to those observed in proved cases of rheumatic fever.

SUMMARY

Three cases are presented which have one common feature, namely, a verrucous aortitis. In one case, which was associated with a stenosis of the isthmus, the verrucous aortitis had caused a spontaneous rupture of the aorta in an eleven-year-old child. In another, a four-year-old child, an aneurysm of the aorta developed on the basis of it and ruptured into the pericardial sac. Streptococci were demonstrated in this case.

The formation of the verrucae is explained on the basis of a fibrinoid swelling and necrosis of the ground substance of the intima with proliferation of the adjacent fibrocytes. The rupture and aneurysm are explained on the basis of necrosis destroying the internal elastic membrane with subsequent weakening of the media. The nonspecificity of the verrucous lesion of the intima of the aorta is pointed out.

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CORYNEBACTERIUM DIPHTHERIAE GRAVIS FOUND IN MARYLAND*

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IN A study of *C. diphtheriae* and similar organisms in Maryland, an organism resembling *C. diphtheriae gravis* described by Anderson, Happold, McLeod, and Thomson[1] was recently isolated. This organism was obtained from throat and nose cultures in three contact cases in a family in which there was a fatal case.

The bacteriologic findings are given in the following description.

Colony Morphology.—The colonies on tellurite agar in 48 hours are flatter and more spreading than those of the ordinary colonies of *C. diphtheriae.* The colonies are also dull grayish black (nonglistening) as contrasted with the glistening black colonies of the ordinary type. They have radial lines which are noticeable at 48 hours and are still more evident later. This is contrasted with the smooth colony of the ordinary type with its more pronounced concentric ridges.

Morphology of Organism.—Grown on Löffler's medium, the organism lacks the slenderness usually associated with the ordinary type and, when stained with methylene blue, the areas which usually stain intensely in the ordinary types take the stain less heavily, so that the general impression is that of a lighter blue. Also few or no metachromatic granules are found. When it is grown on infusion agar slants, the morphology is even more strikingly different from that of the ordinary type. The organism then is much shorter resembling *C. xerosis.* The staining is even and lighter blue, and there is an absence of granules.

Pellicle Formation.—In infusion broth, a pellicle forms after an incubation period of 24 hours. This is easily visible if the tubes are held up to the light and viewed from below, diagonally. There is a granular deposit, and the broth remains clear. After 48 hours' incubation, these observations are decidedly pronounced. The ordinary type shows little or no pellicle, a moderately granular formation and a definite turbidity of the broth.

Hemolytic Action.—There is very little hemolysis in poured blood agar plates after 24 hours' incubation as contrasted with a definite clear zone produced by the ordinary type of *C. diphtheriae,* but on further incubation, definite hemolysis is produced.

Fermentation Reactions.—Acid is produced from dextrose, dextrin, maltose, starch, and glycogen in nutrient broth medium. The ordinary strains do not produce acid from starch or glycogen. Acid is not produced from sucrose.

Virulence.—Virulence was tested intradermally by administering from 0.1 to 0.15 c.c. of suspension of a 24-hour-old growth from a Löffler slant in 5 c.c. of normal salt solution. Three-hundred-gram guinea pigs were used. The con-

*This report represents part of a thesis study on *C. diphtheriae.* The work was done in the Bureau of Bacteriology, Maryland State Department of Health, and the Department of Bacteriology of The Johns Hopkins School of Hygiene and Public Health.

Received for publication, August 24, 1933.

trol guinea pig received 500 units of diphtheria antitoxin 24 hours previous to the time both control and test guinea pigs were inoculated. A very marked reaction occurred in 24 hours in the test guinea pig while little or no reaction was noted in the control animal. A zone of erythema and edema about an inch to an inch and a quarter was produced. Test guinea pigs all died in from 48 to 72 hours. Autopsy revealed primarily congested and very hemorrhagic suprarenals with considerable serous fluid in the pleural and pericardial cavities.

The differences observed in the above described organism are probably due to dissociation of the ordinary type of *C. diphtheriae*. The organism is apparently in the "rough" phase.

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THE SEDIMENTATION REACTION IN THE NEWBORN*

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SINCE 1918 when the sedimentation speed of the erythrocytes was first popularized by Fahraeus, there has gradually grown up a literature relating to this phenomenon, which has now reached considerable proportions. This in the main has been due to the intensive efforts of clinicians and laboratory workers to make use of this characteristic of the red blood cells as a diagnostic and prognostic agent in practically all the conditions which go to make up the field of medicine in its broadest sense. For the most part this work has been confined to adults and it is only within a relatively recent interval that the stimulus for investigative work has been transmitted over to the pediatric field.

A review of the literature quickly impresses one with the fact that a great number of methods have been evolved to obtain the sedimentation speed of erythrocytes. It is because of this variety of methods and the different terms employed in expressing the results that the reader is hard put to compare the sedimentation values as obtained by different authors and is forced to content himself with the conclusions of the author that the sedimentation reaction is normal, increased or decreased in certain conditions, pathologic or otherwise. This lack of standardization may perhaps account for the conflicting statements, as to the sedimentation reaction in normal individuals and in various diseases, appearing in the literature and undoubtedly impairs the value of the test.

Some confusion exists as to the speed of sedimentation of the erythrocytes of the newborn for Nadolny considered the sedimentation reaction to be slowest at the age of two months while György noted the contrary, claiming that

*From the Maternity Service of Lincoln Hospital.
Received for publication, August 28, 1933.

infants over one month showed a faster sedimentation than adults whereas infants under one month showed a definite slowing in the settling of the red blood cells.

The present work was undertaken to investigate the sedimentation reaction in the newborn by the method introduced by Linzenmeir, a method which the author had employed in the study of normal and pathologic conditions in the adult. Normal infants, born of relatively normal parents, with a normal birth history were selected as the subjects of the investigation.

METHOD

Owing to the technical difficulty of obtaining blood from the antecubital vein of the newborn, the superior longitudinal sinus was selected as the uniform site of obtaining the blood from these infants rather than resorting to any micromethod, it being the purpose of this investigation to obtain the sedimentation reaction values in the newborn by a standard method commonly used for adults, namely, the Linzenmeir macromethod.

The infant was securely mummified on to a padded wooden board roughly corresponding to the size of the child, leaving the head as the only movable part of the infant's body. During the act of drawing blood, this part was firmly held by an assistant effectively preventing any movement of the head. The region of the anterior fontanel was made aseptically clean with iodine and alcohol, and a needle with a 2 c.c. syringe attached containing 0.4 c.c. of 5 per cent sodium citrate was inserted into the longitudinal sinus and blood drawn into the barrel of the syringe up to the 2 c.c. mark. The needle used was ¾ of an inch long, 22 gauge and was short bevelled. The balance of the test was carried out in the usual manner of the Linzenmeir method, the time being noted when the erythrocytes reached the 18 mm. mark.

The test was carried out on infants corresponding in age to the various days of the neonatal period.

TABLE I

NAME	AGE IN DAYS	SEDIMENTATION TIME IN HOURS
Baby De L	2	18
Baby R	3	14
Baby S	3	20
Baby B	4	23
Baby R	4	22
Baby C	4	16
Baby A	4	18
Baby MC	5	7
Baby N	5	17
Baby M	6	16
Baby N	7	14
Baby L	8	14
Baby K	9	12
Baby K	10	12

COMMENT

The vena puncture of the superior sagittal sinus to obtain blood for the sedimentation tests may be open to question by some because of the possibility of damage to the substance of the brain, the formation of a blood clot, etc., but

in careful hands it is a safe method. None of the infants used as subjects in this investigation showed evidence of any trauma during the period of observation. It should, of course, be mentioned that directly following the removal of the needle pressure should be exerted over the puncture site to avoid extravasation of blood or clot formation.

With the closure of the anterior fontanel, the difficulties of obtaining blood in infants is increased unless they happen to present some veins sufficiently prominent to permit successful puncture. Therefore, it is natural that a number of micromethods should have been introduced to permit the use of small amounts of blood, not necessarily obtained from a vein, in the sedimentation test. Linzenmeir and Raunert[3] in 1924 reported a micromethod. One drop of blood is obtained from the lobe of the ear or tip of the finger. A capillary tube of 1 mm. width with a dilated reservoir and the upper end supplied with a thick-walled rubber tubing is filled to a certain mark with 5 per cent sodium citrate, and then the blood is aspirated to another mark so that a ratio of 1:4 is obtained. The blood is mixed in the dilated part of the tube and is placed in a rack and sealed with a rubber plate. The sedimentation time is read as in the macromethod.

Numerous other micromethods have been reported in the literature since then by Langer and Schmidt,[4] Cutler,[7] Poindecker,[6] Haselhorst,[5] Kowarski,[8] Ackerman,[9] and others, but all of them depend upon the securing of blood by means of some type of capillary tube. It is generally felt that the micromethod, although slightly less reliable than the macromethod, nevertheless gives sufficiently good clinical results to be of value especially where blood cannot be easily obtained from a vein, as often happens in infants and obese individuals. Investigation has shown that a capillary tube less than 1 mm. in diameter leads to irregular results. Kowarski also calls attention to the fact that blood from the tip of the finger settles somewhat faster than venous blood. This difference can be compensated for by changing the quantitative proportion of citrate solution to the blood, thus, Kowarski uses a dilution of 1:2.5.

DISCUSSION

According to the results obtained in this investigation, the sedimentation time in the normal newborn infant seems to range between seven and twenty-three hours, with the average sedimentation speed for the entire neonatal period being about fifteen hours as compared to the two hours considered to be normal in adults. There is also reflected a tendency for the sedimentation reaction to become less prolonged as the infant grows older.

Silzer[11] in a study of the sedimentation speed of red blood cells from the umbilical vein of 800 infants noted that 91.75 per cent of the cases showed a sedimentation time over twenty-four hours; 4.25 per cent showed sedimentation in from twelve to twenty-four hours; 3.8 per cent in from one to twelve hours and ½ per cent showed sedimentation in from thirty to sixty minutes. That the blood fibrinogen is probably the most important factor in the prolongation of the sedimentation reaction in infants is suggested by the work of Bruchsaler[12] who studied the relative sedimentation reaction and blood fibrino-

gen in infants and pregnant women and found that the maternal blood showed from 250 to 500 units of fibrinogen while the blood of the newborn infants contained from 64 to 125 units.

CONCLUSIONS

1. The speed of sedimentation of the erythrocytes in the newborn is considerably prolonged.

2. There is a tendency for the sedimentation reaction to be less retarded as the infant becomes older.

3. The use of the superior longitudinal sinus as a source of obtaining blood for the study of the sedimentation speed in the newborn is discussed.

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OBSERVATIONS ON THE VASCULAR RESPONSE TO DRAINAGE OF ASCITES*

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IT HAS been observed that venous pressure may fall after excessive abdominal distention has been released. Plumier,[1] in a series of animal experiments, found a drop in the femoral vein pressure after release of fluid previously injected into the peritoneal cavity. Villaret and Saint-Girons[2] noted a temporary fall in venous pressure after ascites was removed from patients with portal cirrhosis. Brams, Katz, and Kohn[3] noted a marked fall in pressure in the femoral vein in animal experiments after abrupt release of abdominal distention. On the other hand, the pressure in the external jugular changed in only a few instances, the fall being much less than in the femoral vein. The carotid pressure in these experiments varied on abrupt release of abdominal distention; a fall, either transient or prolonged, occurred in about two-thirds of the experiments, and a rise was present in the remainder.

The present research was undertaken (a) to study the circulatory responses *during* the removal of ascitic fluid in patients with portal cirrhosis and chronic ascites, and (b) to compare the results of such gradual release of abdominal distention in these patients with the effects of more abrupt release in animal experiments.

The direct method of determining venous pressure was used. A hollow needle was inserted into the median basilic vein and connected with a manometer by suitable rubber tubing. The entire system was filled with 3 per cent sterile citrate solution from a reservoir. A little citrate solution was permitted to flow into the vein through the needle every few minutes in order to prevent clotting. Compression of the veins of the arm was prevented by adducting the extremity. Patency of the needle was tested before and after each reading of venous pressure. Sufficient time was allowed in all instances for stabilization of the level of the citrate solution in the manometer. The level of the right auricle was marked in the fourth right interspace at the junction of the middle and anterior thirds of the thorax and this point was used as the zero level on the manometer. This level was determined before each reading in order to prevent errors due to change in position of the patient during the course of the drainage.

Ten patients with chronic ascites of marked degree due to portal cirrhosis were selected for this study. Three of the patients were in the recumbent position and 7 were sitting during the drainage. Paracentesis was performed in the hypogastrium with a large trocar under aseptic precautions and after a preliminary local anesthesia. A long rubber tube was attached to the trocar in

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Received for publication, August 30, 1933.
Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

order to siphon the ascitic fluid into a receptacle resting on the floor. The fluid was removed in stages, a liter at each stage in 8 experiments and in larger quantities in the other two. Venous pressure, systolic and diastolic arterial pressure, and pulse rate were noted at each stage. Respirations and the general condition of the patient were also closely observed in all instances.

The control venous pressure was normal or slightly elevated in 9 of the patients and low in one. This does not agree with Villaret and Saint-Girons[2] who reported a low venous pressure in all their patients with portal cirrhosis.

TABLE I

PROTOCOL OF CASE 6, TYPICAL OF THOSE OBSERVED IN ALL INSTANCES (FEMALE, AGE FIFTY YEARS; DIAGNOSIS, PORTAL CIRRHOSIS OF LIVER WITH CHRONIC ASCITES; SITTING POSTURE IN BED)

AMOUNT FLUID REMOVED (C.C.)	VENOUS PRESSURE MM. CITRATE	BLOOD PRESSURE MM. HG	PULSE RATE PER MINUTE
Control	108	142/128	132
1000*	105	144/128	108
2000	92	144/128	116
3000	95	142/128	120
4000	80	142/118	120
5000	70	136/112	126
6000	85	134/108	128
7000	72	136/106	116
8000	70	134/104	120
9000	60	132/100	116
10000	60	130/104	124
11000	58	129/102	120
12000	58	129/102	120
13000	65	128/98	108
14000	60	124/98	106
15000	50	132/108	124
16000	55	128/106	116
17000	50	128/98	122
18000	40	120/96	120

*Five minutes elapsed between removal of each 1000 c.c.

The changes in Case 6 are typical of those which occurred in all instances and are shown in Table I. An early fall in venous pressure occurred in all 8 patients in whom determinations were made after the removal of each 1,000 c.c. of ascitic fluid. In four of these the lower level of venous pressure was maintained, while in the remaining 4, a further fall occurred as more fluid was withdrawn. In all of the patients the venous pressure was lower at the end of the drainage than at the beginning.

The systolic arterial blood pressure fell in all but one of the patients, the drop varying from 4 to 34 mm. of mercury. This is in contrast to the variable results noted after abrupt release of abdominal distention in animals.[3] The diastolic blood pressure fell in all instances, the decrease varying from 4 to 32 mm. of mercury. The arterial blood pressure, like the venous pressure, began to fall early in the experiment. Faintness occurred in one instance where the systolic blood pressure fell from 94 to 60 mm. of mercury and the diastolic, from 76 to 54. The venous pressure in his patient had been falling gradually from the beginning, from 80 to 54 mm. of citrate solution. At the

time the patient complained of vertigo, the venous pressure fell abruptly from 54 to 35 mm. of citrate solution. The patient recovered in a few minutes after which the systolic blood pressure rose to 74 and the diastolic to 54 mm. of mercury. The venous pressure also rose at this point to 43 mm. of citrate solution. The fall in arterial pressure in this patient occurred before the venous pressure dropped; a finding in accord with that reported by Brams, Katz and Kohn[3] in animal experiments.

The pulse rate was slower in 6 patients at the end of the experiment and unchanged in 4. It is interesting to note that no increase in pulse rate occurred during the period of faintness in the patient described above in detail.

The early fall in venous pressure appears to be due chiefly to improvement in respiratory excursion. The respirations became definitely deeper after some ascitic fluid was removed. A further fall in venous pressure occurred in these patients when they were instructed to breathe more deeply. A similar fall in venous pressure was reported by Meyer and Middleton[4] during the hyperpneic stage of Cheyne-Stokes respiration and by Kroetz[5] after continuous deep breathing. The mechanism for the venous pressure drop might be the increased aspiration of blood into the thorax accompanying the greater respiratory excursion.

The fall in arterial blood pressure and pulse rate is more difficult to explain. A number of factors were pointed out in the discussion of the results obtained in animal experiments; the most likely factors being (1) decrease in peripheral resistance due to reduction of compression of the abdominal aorta, arterioles, and capillaries and (2) reflexes via the splanchnic nerves.

The clinical significance of a fall in blood pressure is important, as a marked drop such as occurred in some of our patients may lead to collapse. In a patient with a normal blood pressure and a quickly adjusting vasomotor reaction, no harm may result but even a small blood pressure drop may be serious if the blood pressure is low to begin with. A subsequent study of all 10 cases showed that the only patient in whom collapse occurred was in an instance where the blood pressure before tapping was 94/78. Further observations are necessary to determine whether such a low blood pressure warrants caution in the removal of ascites. Another patient (Case 3) went into collapse on two preceding occasions when the fluid was removed while in a sitting posture. His systolic blood pressure was 142 mm. of mercury and the diastolic was 90. Removal of the same quantity of ascitic fluid at the same rate as on the previous occasions resulted in no untoward effects when drainage was performed in the supine position. It is possible that this patient with a normal blood pressure is an example of failure of the vasomotor system to adapt itself sufficiently to the changes induced by removal of ascites. In any event, it is suggested that the patient be placed in a recumbent position in all cases where ascites is to be drained, and a hypodermic syringe of adrenalin be available.

SUMMARY

1. The effect of draining off abdominal fluid on the venous pressure, systolic and diastolic arterial blood pressure and pulse rate was studied in 10 patients with portal cirrhosis and chronic ascites.

2. The venous pressure began to fall early during the drainage, and in four instances continued as more fluid was removed. The venous pressure at the end of the drainage was lower in every instance than before tapping was begun.

3. Systolic and diastolic arterial pressures fell while the fluid was being removed. A fall in blood pressure was associated with faintness in one patient whose pressure was low before the procedure was begun. A similar fall in other cases with previously normal blood pressure was not associated with such untoward symptoms.

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PIGMENT STUDIES*

I. On a Brown Skin-Adrenalin Color Reaction

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MEIROWSKY[1] seems to have been first to call attention to the fact that a melanin-like substance forms itself when an extract of human skin is heated in the presence of adrenalin. His original experiment was made with a mixture that consisted of "suprarenin (1:1,000)" one part, extract of preputial skin one part, and water one part. After this preparation had been incubated for twenty-four hours at 56° C., he noted that its color had changed from a light yellow to an intense grayish black. More recently[2] it has been shown that sections of whole skin exhibit a like reaction.

Two questions about this striking phenomenon still await elucidation—one concerns the chemical composition of the pigment substance itself and the other, its mode of formation.

With respect to the second query—the only one considered here—the view seems to prevail very generally that the pigment is produced in some way through the activity of an oxidizing ferment present only in living epithelial cells. Indeed, having shown experimentally that sections of human skin treated with a 1 to 2 per cent aqueous solution of 3, 4-dioxyphenylalanin exhibit a similar color change. Bloch[3] insists that the brownish substance is formed through the agency of a specific intracellular ferment, the so-called

*Received for publication, August 30, 1933.

dopaoxydase. This ferment is said to be thermolabile[3] and to be rendered inactive, if not actually destroyed, at a temperature of 57° C.

The work herein reported was undertaken in order to ascertain, in regard to the factor of temperature whether sections of human skin would display a peculiarity previously noted in experiments with a finely powdered preparation of ricinus lipase. Concerning this peculiarity, it was found constantly on incubating a small quantity of the lipase material in a dilute solution of adrenalin at 45° C. that in the course of a week or two the color of the precipitated powder gradually changed from white to brownish black. But, on the other hand, it was also noted constantly that this color reaction took place with equal, if not slighter, intensity when such experiments were repeated with a lipase powder previously sterilized by heating it in steam for half an hour at 100° C. To check this finding by means of another tissue, similar experiments were made with bits of human skin, using as test objects small squares of it that measured about 5 mm. on a side.

MATERIAL AND TECHNIC

The working material used was obtained, as needed, from 26 selected seemingly normal, coroner's subjects. Qualitative details about this material may be dispensed with, as the various specimens of skin tested did not seem to differ from one another significantly as regards their pigment-forming capabilities. It should be stated, however, that care was taken to select skin that was both white in color and free from coarse hairs. Each specimen was prepared for use by paring away as much as possible of the subcutaneous fat. This having been done, and the skin having been washed clean in running tap water and dried with a cloth, it was then cut into squares of the proper size.

It was found convenient to make the experiments in small test tubes (1 cm. in diameter). The preparation used routinely consisted of a single test square and 4 c.c. of an adrenalin solution made by diluting Parke, Davis and Company's adrenalin solution (1:1,000) with freshly boiled distilled water in the proportion of 1 to 5. Whether fresh or steamed, the squares were tested in three ways, namely, in an open tube, in another sealed by a layer of heavy mineral oil and in a third tube sealed by fusing the open end over a Bunsen burner. Toluene was added only to the unsealed preparations. The tests were set up in parallel series. In one of these the skin was tested in its original, fresh state, in the other, after it had been heated for half an hour in moist

TABLE I*

PIGMENT FORMATION IN HYDROLYZING SKIN-ADRENALIN SYSTEMS

MATERIAL TESTED	PREPARATIONS		
	OPEN	SEALED UNDER OIL	SEALED BY FUSION
Squares of fresh skin	73	60	104
Squares of steamed skin	58	58	67

*Shows average time interval, in hours, before appearance of a brown substance in preparations made, respectively, with squares of living skin and squares of devitalized skin. Values shown are based on data noted in parallel series of experiments with skin specimens obtained from 26 coroner's subjects. Incubation temperature 45° C.

steam at 100° C. Satisfactory results were obtained by incubating the preparations at a constant temperature of 45° C.

RESULTS

When such preparations were placed in the incubator and observed from day to day, in the course of from twenty-four to seventy-two hours it was noted that the adrenalin solution became faintly pink in color; and that immediately after this coloring matter made its appearance—never before—the skin squares began to turn brown. This change was at first barely perceptible as an extremely pale shade of brown. But the reaction being a progressive one, often in the course of from six to ten days, the squares became almost black in color. There seemed to be a relationship of some sort between the rate of formation of the brown pigment and the color of the supernatant adrenalin solution. For it was obvious that the brown material was produced but slowly while the adrenalin solution was pale pink in color, at a more rapid rate while it exhibited a clear, rose pink shade, and most rapidly when its color had become reddish brown. These color changes occurred earlier and seemed to be somewhat more pronounced in the preparations made with squares of devitalized skin than in those made with squares of living skin; the average values obtained as regards the time of onset of the brown staining effect were, respectively, fifty-eight hours and seventy-three hours (Table I). Formation of the brown dyestuff took place in both of the preparations from which air was excluded. But on the whole it formed more slowly in the preparations sealed by fusion than in those sealed by a layer of mineral oil.

In numerous collateral series of experiments it was noted, constantly, that the addition of 1 per cent by weight of sodium chloride to a preparation caused the square—whether a heated or an unheated one—to turn brown within twenty-four hours and to exhibit a maximum grade of pigmentation within a period of four days.

Control squares of skin were incubated in distilled water at 45° C. Without exception, after an incubation period of two weeks each square remained as perfectly white as it had been when in the fresh state.

SUMMARY

1. Experimental data are presented which show that bits of human skin become dark brown in color on being incubated in a dilute solution of adrenalin.

2. The results obtained seem to show conclusively that the brown pigment forms itself in the absence of any organized ferment.

The author wishes to thank Mr. Paul C. Greene for valuable technical assistance rendered during the course of this study.

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2512 Washington Street

PIGMENT STUDIES*

II. On the Importance of Hydrolyzed Adrenalin as a Tissue Stain

Clarence Quinan, M.D., San Francisco, Calif.

IN THE preceding study it was found with respect to two organic substances, one of vegetable, the other of animal origin, that they gradually became almost black in color if they were incubated for some time at 45° C. in a hydrolyzing solution of adrenalin; and that this phenomenon occurred whether these substances were tested in the fresh state or after they had been heated in live steam for half an hour at 100° C. The materials tested were small quantities of defatted castor bean meal and bits of human skin.

Apparently, the conditions under which the experiments had been made were such as to preclude the possibility that the melanin-like pigment had formed itself through the agency of an organized ferment. It seemed probable, nevertheless, that the process of pigment formation was essentially a catalytic one; and that the color change noted took place because of the fact that the solution contained some cleavage product of adrenalin which acted upon the tissues and dyed them brown. In other words, the evidence seemed to indicate that the solution of hydrolyzed adrenalin acted simply as a non-specific stain. As it seemed worth while to secure, if possible, some data on this point, many series of experiments were made with suitable test objects representative of various cellular structures. The results obtained show that under proper conditions a hydrolyzing solution of adrenalin is capable of imparting an intense brownish black color to a number of organic substances. Since it does not appear to be generally known that such a solution has tinctorial properties, this paper gives a brief account of the staining experiments.

MATERIAL

Four kinds of material were tested: white holly, normal human skin, normal human red bone marow, and blood obtained from a patient afflicted with myelogenous leucemia.

The white holly was purchased from a cabinetmaker. It consisted of small sheets of veneering, about 1 mm. in thickness and perfectly "dressed" on both surfaces. Pieces of this wood 1 cm. square were used in the experiments. The specimens of skin tested, as well as those of bone marrow, were obtained, as needed, from carefully selected coroner's subjects. The tissues were prepared for use as follows: Each skin specimen, in the usual manner, was first hardened in 10 per cent formaldehyde and then embedded in paraffin and sectioned. The staining tests were made with sections attached to glass slides by means of an albumin fixative. In the experiments made, respectively, with red bone marrow and leucemic blood, the usual smear preparations were used. Both were fixed in absolute alcohol.

*Received for publication, August 30, 1933.

TECHNIC

1. Preparation of the Staining Solution.—Into a cylindric specimen tube 3 cm. by 8 cm. in size, 3 c.c. of Parke, Davis and Company's adrenalin solution (1:1,000) and 7 c.c. of freshly boiled distilled water were measured by means of a pipette and then, to serve as a catalytic agent, a bit of human skin 5 mm. square—previously steamed for half an hour at 100° C.—was dropped into the tube. To prevent loss by evaporation the tube was provided with a tightly fitting stopper. The preparation then was incubated at 45° C. and observed from day to day with reference to the appearance of two color reactions—one in the supernatant solution, the other in the little piece of tissue. As a rule, in the course of from one to three days, the solution gradually became deep rose pink in color while, toward the end of that period, the bit of skin began to exhibit a very pale brownish discoloration. When both of these color changes were visible, the solution was ready for use.

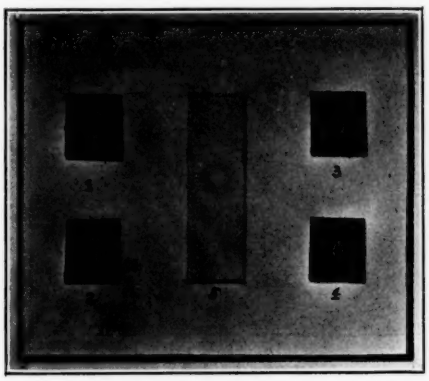

Fig. 1.—Squares of white holly stained in a solution of hydrolyzing adrenalin. One and three are squares of the wood in its natural, unheated state. Two and four are squares steamed for half an hour at 100° C. before they were tested. Five is the control strip. Incubation period was six days. Temperature 45° C. Note that the heated squares are darker in color than the unheated ones.

2. The Staining Procedure.—Slide preparations of the tissues to be tested were brought into the rose pink solution and then incubated at 45° C. until the desired brown staining effect developed. This usually required a period of from six to ten days. It should be mentioned that the squares of white holly were introduced into the solution edgewise and kept in a vertical position by means of suitable holder devices fashioned out of glass tubing.

RESULTS

White Holly

Experimental evidence was sought especially in regard to two points: (1) Does heat modify the action of the stain in any way? (2) Is the staining process modified by the factor of concentration?

Influence of the Heat Factor.—Method: Two pieces of white holly were used in each experiment, namely, a square of the wood in its natural, unheated state, and a square that had been steamed for half an hour at 100° C.

Results: On removing the squares from the solution after the proper time interval, it was found constantly (1) that both of the squares had changed in color from white to brown; (2) that the heated square was several shades darker in color than the unheated one. (See Fig. 1.)

Influence of the Concentration Factor.—Method: Proceeding in the manner already described and using in each instance a bit of steamed skin as the catalyst, a series of five staining solutions, regularly graded in concentration according to the amount of adrenalin present in each one, were made ready for use in the small cylindric containers. By volume these preparations contained, respectively, 20, 40, 60, 80, and 100 per cent of commercial adrenalin solution (1:1,000). Having then placed in each container a square of unheated white holly to serve as a test object, the preparations were incubated at 45° C. for the usual length of time.

Fig. 2.—Showing influence of the concentration factor. The unheated squares, one to five were stained simultaneously in solutions that contained, respectively, 20, 40, 60, 80, and 100 per cent of adrenalin (1:1000). Incubation period six days. Temperature 45° C.

Results: In a typical experiment, after the lapse of six days it was found that the set of squares displayed the color changes of graded intensity shown in Fig. 2. The tone range was from pale brown to brownish black. The differences between individual squares of a series, as regards the relative depth of the staining effect, were rendered remarkable by a particular circumstance; for it will be noted that the grain of the wood stands out more or less conspicuously in the first three squares, stained in the weaker solutions, while the remaining two squares appear to be uniformly black in color.

Skin

Hope was entertained that suitable tests with thin sections of skin would serve to explain a peculiarity in regard to the distribution of the brown dyestuff previously noted in the experiments with whole skin. For on examining bits of blackened skin removed from old testing solutions, it had been observed invariably that the brownish black staining effect was most pronounced at or near the free epithelial surface of the specimen while, in striking contrast, the deeper, subcutaneous tissues showed much lighter degrees of discoloration. Since it seemed probable that thin sections of skin would stain more

evenly, numerous slide preparations were incubated in the staining solution until the proper color developed and then were examined under the microscope. Contrary to expectation, however, it was found in every case: (1) That the amount of the brown material present in the tissue increased in the direction from the depth toward the free surface of the section. (2) That the deep layers of the stratum corneum were so intensely stained that an average section (see Fig. 3) had the appearance of being bordered on one side by a sharply defined zone wherein the black staining effect closely resembled that observed in the experiments with white holly.

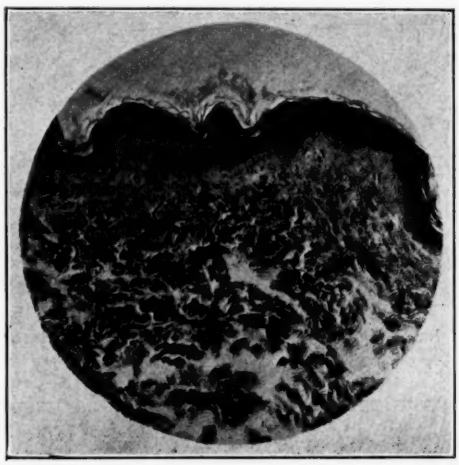

Fig. 3.—Section of normal human skin stained in hydrolyzed adrenalin for six days. (×125)

Comment: The foregoing observations seem to justify the inference that the depth of the brown discoloration at any given level in a stained section of skin is related in some way to a density factor. At any rate, figuratively speaking, it would appear that somewhat as the lines in a contour map indicate the pitch of a slope by their nearness to one another, so in a stained section of skin the color appears to be darkest near the surface where the cells are closely packed.

Leucemic Blood and Red Bone Marrow

Method: As it was desired to find out what staining effects, if any, would take place if representative groups of leucocytes were incubated in the adrenalin solution, simultaneous tests were made with two smear preparations—one of leucemic blood and one of bone marrow. Twenty experiments were made in all. The average time period was six days.

Results: Although the pigmentary changes observed varied considerably both in degree and extent, it was found, without a single exception, that the leucocytes exhibited brown color reactions. In a majority of the specimens the staining effects were comparatively slight in degree and were limited to the nuclei, causing these to appear as pale brown objects sharply outlined within the colorless cell bodies. But not a few other specimens were encoun-

tered (see Figs. 4 and 5), which afforded obvious proof that the brown dye had stained deeply not only the nuclei of the leucocytes but also the myelocytic granules. Therefore, having in mind the evidence previously acquired, it was inferred from these observations, (1) that the test solution contained a diffusible nonspecific pigment of unknown composition; and (2) that al-

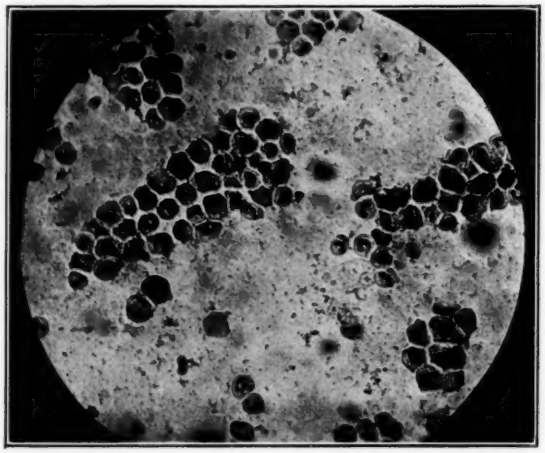

Fig. 4.—Smear of leucemic blood stained in hydrolyzed adrenalin for six days.

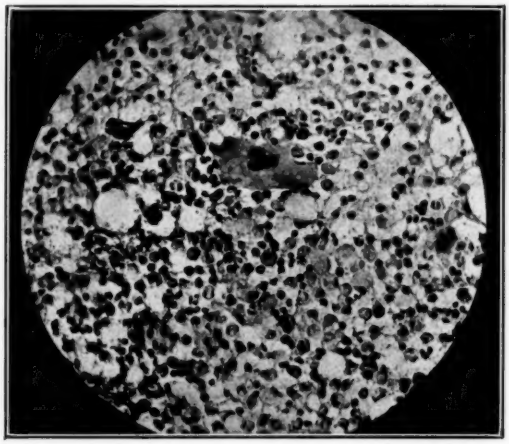

Fig. 5.—Smear of human red bone marrow stained in hydrolyzed adrenalin for six days.

though the conditions essential to the development of its maximum activity were undetermined, this substance stained the component tissues of the leucocytes with unequal intensity.

SUMMARY

1. A new method is presented for the preparation of a rose pink solution of hydrolyzed adrenalin.

2. Under suitable conditions this solution imparts a deep brown or brownish black color to various organic substances.

3. The brown dye appears to be nonspecific in its action.

4. The staining effects obtained with specimens of white holly, human skin, leucemic blood and red bone marrow are described.

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CRYSTALLINE ELEMENTS IN THE STOMACH LAVAGE OF PATIENTS WITH CHOLELITHIASIS*

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IN A PREVIOUS paper,[1] certain types of crystals, present in preoperative specimens of bile, in patients with gallbladder disease, were described and compared with biliary crystals found in the scrapings of stones and bile removed from the gallbladder at operation. During the course of the above investigation, it was observed that in some patients, the duodenal tube did not pass beyond the pylorus within a period of eighteen hours. A microscopic examination of the aspirated gastric contents of these patients revealed various types of biliary crystals. It was, therefore, thought of interest to determine the frequency with which crystalline elements were found in the gastric contents of patients with gallbladder disease. For this study, 28 patients were observed.

The patients were grouped as follows: Group 1, consisted of 21 patients, in whom there was definite roentgenographic evidence of cholelithiasis. Group 2 (the control group), consisted of 7 patients who were free from gastrointestinal symptoms.

The gastric contents of all the patients were obtained by stomach lavage, because (lavage) water was found to be the best medium for the microscopic detection of biliary crystals. The gastric lavage was performed with plain tap water, preferably in the morning, on any empty stomach. A specimen of the lavage water was then collected and examined microscopically.

In Group 1, there were 21 patients. Five of these were males, and 16 females, the ages ranging from thirty-one to sixty years. Typical and atypical cholesterol crystals, carbonate crystals, and calcium bilirubinate pigment (granular and amorphous types), separately or collectively, were found in the gastric lavage of every one of the patients in this group. The crystals and pigment were present in appreciable amounts, and as follows: Cholesterol and calcium bilirubinate pigment, together in 14 patients; cholesterol and carbonate crystals together with calcium bilirubinate pigment in 3 patients (Figs. 1 and 2); cholesterol crystals alone in 2 patients; and calcium bilirubinate pigment alone in 2 patients. The cholesterol type of crystal predominated in 9 of the patients in this group. Calcium bilirubinate pigment was most abundant in

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Received for publication, September 5, 1933.

12 patients. The crystalline elements were found, at times, in the mucus, and, at other times, independent of the mucus. It was not necessary to centrifuge the specimens to obtain the crystals.

Fig. 1.—Mrs. K. Cholesterol and carbonate crystals; slight amount of calcium bilirubinate pigment. (Microphotograph of particle in preoperative specimen of gastric lavage.)

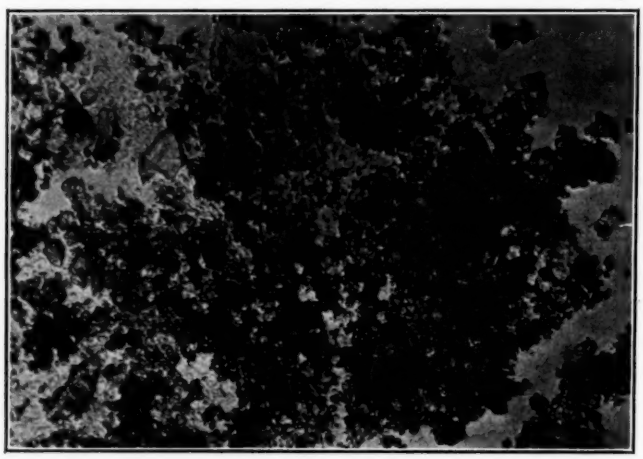

Fig. 2.—Mrs. F. Abundant amount of calcium bilirubinate pigment and few cholesterol and carbonate crystals. (Microphotograph of particle in preoperative specimen of gastric lavage.)

Eighteen of the patients in Group 1 were operated upon. In all of these cases, the crystalline elements observed in the lavage water, preoperatively, were found to be similar in appearance to the biliary crystals seen in the scrapings of stones or bile removed from the gallbladder at the time of operation.

Group 2 (the control group) consisted of 7 patients ranging in age from twenty-five to fifty years, all of whom were free from gastrointestinal symptoms. Four of these patients were males, and 3 were females. These patients came to the hospital for other than abdominal ailments, and, as far as could be ascertained, never complained of gastric symptoms. Microscopic examination of the stomach lavage obtained from these patients did not reveal the presence of cholesterol or carbonate crystals. In only 4 of the patients a slight amount of calcium bilirubinate pigment was found.

COMMENTS

Cholesterol and carbonate crystals and calcium bilirubinate pigment were found, separately and collectively, in appreciable amounts, in the stomach lavage of a group of patients suffering from calculous cholecystitis. The presence of these crystalline elements in the gastric lavage was due to regurgitation of the bile and duodenal contents into the stomach. In about one-half of the patients the gastric lavage water was bile tinged, and, in the remaining patients, it was colorless.

Although biliary crystals were present in the stomach lavage in appreciable amounts, they were not, as a rule, as numerous as the crystals seen in preoperative specimens of bile of patients with cholelithiasis. When, however, the gastric lavage was performed within six hours after the onset of an attack of biliary colic, showers of crystals and an abundant amount of calcium bilirubinate pigment were found.

Carbonate crystals in the stomach lavage were dissolved by hydrochloric acid which aided in differentiating these crystals from the atypical types of cholesterol crystals. At times, starch granules in the lavage water had to be distinguished from biliary crystals. This was readily done by the addition of iodine.

Microscopic examination of the stomach lavage of the patients in the control group did not reveal any cholesterol or carbonate crystals; yet, a slight amount of calcium bilirubinate pigment was found in four of the patients. In a previous paper,[1] it was emphasized that a few cholesterol crystals or a small amount of calcium bilirubinate pigment, present in preoperative specimens of bile, had no pathologic significance. The presence of a slight amount of calcium bilirubinate pigment in the stomach lavage, likewise, cannot be regarded as abnormal.

The presence of biliary crystals in the stomach lavage of patients with cholelithiasis, is of some significance. However, an exact diagnostic appraisal of this finding cannot as yet be given, as this subject is still under investigation.

CONCLUSIONS

1. A series of 21 patients with cholelithiasis, and another 7 patients (control group) free from gastrointestinal symptoms, were subjected to a microscopic examination of the gastric contents obtained by lavage.

2. The stomach lavage of the patients with calculous cholecystitis showed appreciable amounts of cholesterol and carbonate crystals and calcium bilirubinate pigment occurring separately and collectively.

3. In the control group the gastric lavage showed, in only four patients, a slight amount of calcium bilirubinate pigment, which is not regarded as abnormal.

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EFFECT OF SMOKING ON SKIN TEMPERATURE*

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CONSIDERABLE investigation has been carried out during the past decade in an attempt to determine the various effects of tobacco on the human body. It has long been thought that tobacco was an important contributing etiologic factor in some of the vascular diseases, most notably thromboangiitis obliterans. Certain other conditions also have been so frequently associated with the practice of smoking that the causal connection has been considered indisputable by some authorities. In this group we can mention cardiac arhythmias, shortness of breath, and chronic congestion of the nasopharynx.

On chemical analysis of tobacco smoke, Bogen[1] points out that the composition of the smoke obtained from any cigaret depends to a great extent on the manner and rate with which the cigaret is smoked. The sidestream, or the smoke from the lighted end of the cigaret, ordinarily constitutes the greater part of the actual materials given off from the combustion. This contains a low concentration of carbon monoxide with a relatively high percentage of nicotine. Smoke which has been sucked in contains an appreciable amount of carbon monoxide. This has been estimated as high as 8.3 c.c. from each gram of tobacco smoked, which is approximately the amount of tobacco in each cigaret. The amount of nicotine in the smoke sucked in is much greater than that of the sidestream. This has been estimated at from 3 mg. to 9 mg. from each gram of tobacco smoked, and 88 per cent of this is absorbed. Bogen notes also that domestic cigarets are somewhat richer in nicotine than many of the imported brands. The domestic brands average about 2.5 per cent nicotine.

Whether or not nicotine is the primary toxic agent in tobacco smoke, it is interesting to note that according to Bastedo[2] nicotine is rapidly absorbed from skin and mucous membranes. Its main action is a brief stimulation of the sympathetic and parasympathetic ganglia, followed by a depression. The stimulation of the ganglia results in a rise in blood pressure. The lethal dose of nicotine for an adult is about 500 mg. In addition to the nicotine and carbon monoxide, the aspirated smoke contains pyridine, aldehydes (especially furfural), and volatile oils. These are generally considered of relatively little significance.

That smoking has an effect on the cardiovascular system has long been

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Received for publication, September 22, 1933.

suspected. Simici and Marco[3] in 1926 stated that tobacco smoke produces in both smokers and nonsmokers well-marked disturbances in the cardiovascular system. These disturbances, they consider, are characterized principally by phenomena of vasoconstriction which appear first in the visceral, and then in the peripheral vessels. They found a rise in blood pressure of 20 to 30 mm. of mercury, with an acceleration of pulse of 10 to 40 per minute. They explain this on the basis of a vasoconstriction.

Bruce Miller and Hooker[4] in 1909 believed that there was probably a peripheral vasoconstricting effect from smoking tobacco and demonstrated this by noting the volume changes in the arm and hand. Moschcowitz[5] in 1928 stated that the most probable explanation of the so-called tobacco angina is that it is due to the constrictive action of tobacco on the blood vessels of the heart.

Madoch and Coller[6] have more recently shown what they consider further evidence of a peripheral vasoconstriction as a result of smoking, by determining the temperature of the skin of the upper and lower extremities. In the case mentioned in their report, they found a drop of 5.9° C. in the upper extremity and 1.5° C. drop in the lower extremity while the subject smoked 3 cigarets.

In the present study 34 subjects were used. An attempt was made to obtain a record of the changes in the temperature of the skin while the subjects were smoking. The region just below the nail of the third finger of the left hand was selected as the site for temperature determination. The subject's hand was placed in a comfortable position and relaxed. The instrument used for measurement was the Leeds and Northrup electric recorder. The thermocouple was fastened to the site selected and the record taken.

After a constant temperature was obtained the subject was instructed to smoke a cigaret in his accustomed manner, continuing to smoke until approximately ¾ of the cigaret had been consumed. Several of the popular brands were used. Room temperature varied between 63° F. and 86° F. In no case did the lowest temperature reached go below room temperature during the progress of the experiment.

In Table I 26 of the subjects were habitual smokers, consuming from 15 to 30 cigarettes daily. Of these, 22 showed a drop in skin temperature averaging 4.3° F., 2 showed no change at all, and one showed an elevation of 8° F.

Four of the subjects in Table II were retested, and in each case a comparable change was noted except one, where the drop was approximately half of the original.

In the group of 8 nonsmokers, 3 showed an average drop of 3.2° F., 4 showed no change at all, and 1 showed an elevation of 1° F.

Four cases of thromboangiitis obliterans were studied, 3 of which showed an average drop of 8° F. One showed no change at all.

In practically all of the cases studied where a drop in temperature was noted, the temperature change was evident before one-half the cigaret had been consumed. The average time required to reach the lowest temperature from the beginning of smoking was fourteen minutes, with five minutes as the minimum and fifty minutes as the maximum. The time required to return to the initial temperature or higher after the low point, averaged twelve minutes with five as the minimum and twenty-five as the maximum.

TABLE I

HABITUAL SMOKERS

SUBJECT	ROOM TEMP.	INITIAL TEMP.	LOWEST TEMP.	DEGREES DROP	TIME OF DROP	RETURN TEMP.	TIME OF RETURN	USUAL DAILY CONSUMPTION OF CIGARETS
1	63	75.0	72.5	2.5	12	91.0	14	20
2	63	85.0	80.6	4.4	8	86.0	5	20
3	72	79.0	76.0	3.0	10	83.0	20	15
4	72	81.0	78.0	3.0	12	81.0	6	15
5	68	71.0	67.6	3.4	5	70.0	15	30
6	68	94.4	92.6	1.8	5	94.5	8	30
7	70	87.0	76.0	11.0	20	78.0	5	15
8	72	93.0	88.0	5.0	8	92.0	10	15
10	68	86.0	79.5	6.5	12	87.0	8	15
11	68	87.0	81.5	5.5	17	89.0	14	15
12	80	87.0	81.5	5.5	26			30
13	76	91.0	84.0	7.0	24			20
14	81	98.0	95.5	2.5	6	98.0	8	25
15	89	97.0	93.0	4.0	20	97.0	10	25
17	76	96.0	89.0	7.0	25	95.0	22	30
18	87	98.0	98.0	0.0	20			20
19	80	89.0	87.0	2.0	10	89.0	12	15
22	76	88.0	83.0	5.0	22			15
23	76	86.0	75.0	11.0	50			15
24	86	98.0	96.0	2.0	10	98.0	25	20
26	80	95.0	93.0	2.0	6	96.0	7	25
27	76	98.0	96.0	2.0	5	98.0	10	10
31	80	98.0	94.0	0.0	10	98.0	20	15
32	72	80.0	88.0	+8.0	10			10
33	72	92.0	91.0	2.0	18			15
34	82	86.0	86.0	0.0	15			5

TABLE II

HABITUAL SMOKERS TESTED AT DIFFERENT TIMES

SUBJECT	ROOM TEMP.	INITIAL TEMP.	LOWEST TEMP.	DEGREES DROP	TIME OF DROP	RETURN TEMP.	TIME OF RETURN
					min.		min.
1	63	75	72.5	2.5	12	91	14
	67	85	80.6	4.4	8	86	5
2	72	79	76.0	3.0	10	83	20
	72	81	78.0	3.0	12	81	6
5	70	87	76.0	11.0	20	78	5
	72	93	88.0	5.0	8	92	10
7	68	86	79.5	6.5	12	87	8
	68	87	81.5	5.5	17	89	14

TABLE III

NONSMOKERS

SUBJECT	ROOM TEMP.	INITIAL TEMP.	LOWEST TEMP.	DEGREES DROP	TIME OF DROP	RETURN TEMP.	TIME OF RETURN
						min.	min.
9	66	88	86.0	2.0	6	91	5
16	79	96	97.0	+1.0	20		
20	83	90	86.5	3.5	20	92	18
21	86	98	98.0	0.0	14		
25	81	97	97.0	0.0	50		
28	97	100	100.0	0.0	20		
29	88	98	94.0	4.0	8	97	12
30		98	98.0	0.0	10		

In 4 cases the minimum temperature was reached and the return elevation of temperature began while the subject was still smoking.

There was apparently no relation between the number of cigarets consumed daily by the subject and his temperature reaction.

TABLE IV

THROMBOANGIITIS OBLITERANS

SUBJECT	ROOM TEMP.	INITIAL TEMP.	LOWEST TEMP.	DEGREES DROP	TIME OF DROP	RETURN TEMP.	TIME OF RETURN
					min.		min.
13	76	91	84	7	24		
17	76	96	89	7	25	95	22
23	82	86	75	11	50		
36	80	86	86	0	15		

In comparing the group of smokers with the nonsmokers, it will be noted that 88 per cent of the smokers showed an average drop in skin temperature of 4.3° F. In the nonsmoker group only 36 per cent showed a drop and this average was 3.2° F. Conditions were identical for both groups. It was noted, however, that the nonsmokers did not inhale. Earp[7] has pointed out that inhalers take 8 times as much nicotine into their systems as smokers who do not inhale. Winterstein and Aronson showed that from 2.5 per cent to 4.4 per cent of the nicotine is absorbed on smoking without inhaling and from 8.1 per cent to 17 per cent is absorbed when inhaled. When we consider that in the act of inhaling, the smoke is exposed to the mucous membranes of the nasopharynx and the lower respiratory passages, thus giving an infinitely larger surface on which the products of smoking may condense, we may expect a greater deposition and consequent absorption of nicotine and other components, than would result from simply puffing the smoke in and out of the mouth. This may be the explanation for the marked difference between the temperature changes of the smokers as compared with the nonsmokers.

In personal communication with Dr. Wright, pertaining to his work on capillary activity during active smoking, he states:

"In certain individuals we have been able to note a marked slowing and often stasis in the capillary circulation at the nailfold during the smoking of a single cigaret. This phenomenon when present can be seen after the first few deep inhalations. It has been found especially in subjects who complain of systemic symptoms from the use of tobacco."

COMMENT

As has been repeatedly contended during the past few decades, this work further tends to confirm the opinion that smoking does bring about a peripheral vasoconstriction, which may be a significant contributing etiologic factor in certain of the vascular disorders and particularly in thromboangiitis obliterans.

NOTE: Since this manuscript was prepared, Haggard and Greenberg[8] have shown that an increased blood sugar results from smoking, and they attribute this to adrenal stimulation by nicotine through the sympathetics. This has been confirmed by the present writers[9] who also believe that the vasoconstriction described above can probably be attributed to the same cause.

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VITAMIN THERAPY IN PULMONARY TUBERCULOSIS*

VI. The Effect of Viosterol on the Carbon Dioxide Content, the H-ion Concentration, Chlorides, Glucose and Urea Nitrogen of the Blood, and Protein, Calcium, and Phosphorus of the Serum. The Effect of Physiologic Saline on These Constituents During the State of Hypercalcemia.

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IN PREVIOUS experiments[1] we demonstrated the effect of viosterol on the calcium and phosphorus metabolism, and the effect of intravenous physiologic saline upon these two elements. In order to determine the effect of viosterol upon other constituents of the blood we performed simultaneously the following chemical analyses before, during, and after hypercalcemia. Likewise, the same procedures were employed in determining the effect of physiologic saline upon these constituents when administered during hypercalcemia. These analyses were performed on 15 patients who had a diagnosis of pulmonary tuberculosis, Stages II and III.

METHODS

Serum calcium was determined by the Clark and Collip[2] method, modification of Kramer and Tisdall.[3] Phosphorus determinations were made by the method of Benedict and Theis.[4] McDonald's[5] modification of Hastings and Sendroy's[6] method was used in making the analyses of the P_H. Carbon dioxide was determined by the volumetric method of Van Slyke;[7] urea nitrogen according to the method of Myers;[8] glucose by the micromethod of Folin;[9] chlorides by the method of Whitehorn;[10] and albumin and globulin by the method of

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Received for publication, September 22, 1933.
Grateful acknowledgment is due Miss Grace Heimann for her valuable assistance.

Wu and Ling[11] and Greenberg,[12] respectively. If the albumin determination is allowed to incubate four or five hours, very little difference in the result is obtained, whereas the globulin may vary considerably after two hours.

THE EFFECT OF VIOSTEROL ON SERUM CALCIUM AND PHOSPHORUS

Data presented here further corroborate the earlier work[13, 14] with viosterol 250D* and 10,000X.† The solution used in this experiment had a cod liver oil coefficient of 10,000, which is one hundred times stronger than 250D. The daily dose was 0.5 c.c. (20 gtts.) which is the rat assay equivalent in vitamin D of 5,000 c.c. of cod liver oil. The average period of viosterol administration for this group was approximately eight days. A condition of hypercalcemia as a result of this dosage was brought about between the eighth and fifteenth days. Serum calcium concentrations averaged from 13.3 to 18.4 mg. per 100 c.c. The average normal serum calcium concentration was 11.4 mg. per 100 c.c. for the 15 cases (Table I). The average serum calcium after the period of viosterol administration was 15.2 mg., an average elevation of 3.8 mg. per 100 c.c. The average serum phosphorus concentration was 4.7 mg. per 100 c.c. before, and 4.9 mg. per 100 c.c. after the viosterol administration. It is significant to note that this concentration of phosphorus in the blood serum would have been elevated had it been determined during the first five days of viosterol administration.[14] However, if one determines the serum phosphorus after the serum calcium has reached a higher value, the serum phosphorus is reciprocally lower.

We have demonstrated[1] that physiologic saline lowers the concentration of calcium and phosphorus in the blood, and this work further verifies this specificity. Within twelve hours after the administration of between 3,000 and 5,000 c.c. of physiologic saline per individual, the serum calcium concentration dropped to 12.7 mg. per 100 c.c. (Table I), an average decrease of 2.5 mg. Serum phosphorus was decreased by physiologic saline as shown by the average of 3.7 mg. per 100 c.c., a decline of 1.2 mg.

The lowering of serum calcium and phosphorus is not due to a change in blood volume. Since determinations were made twelve hours after the administration of saline, blood volume would have returned to normal. Smith and Mendel[15] injected into the veins of rabbits isotonic solutions of various neutral sodium salts. The blood volume was doubled although they found that different salts were eliminated from the blood at different rates. Most of the fluid in every case passed into the tissues within five minutes without causing evident edema. If the decrease in serum calcium and phosphorus was due to dilution of the blood volume, a return to the original high concentrations would be expected after the effect of saline had passed.

THE EFFECT OF VIOSTEROL ON P_H AND CO_2

The average P_H for the 15 cases was 7.39 prior to the administration of viosterol. This average is 0.02 more than the P_H of 50 normal individuals who equaled 7.37.[16] After viosterol the average P_H of the blood for the 15 cases was 7.41. The effect of a viosterol hypercalcemia upon the hydrogen ion concen-

*10,000 International units per gram.

†10,000 times average cod liver oil, 1,000,000 International units per gram; kindly supplied by Mead Johnson and Company.

TABLE I

CASE	SERUM												BLOOD											
	CALCIUM MG. PER 100 C.C.			PHOSPHORUS MG. PER 100 C.C.			PROTEIN GM. PER 100 C.C.						CARBON DIOXIDE COMBINING POWER			P_H			CHLORIDES AS NACL MG. PER 100 C.C.			GLUCOSE MG. PER 100 C.C.			UREA NITROGEN MG. PER 100 C.C.		
							GLOBULIN			ALBUMIN																
	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†	NORMAL	AFTER VIOSTEROL* 10,000 X	AFTER NACL†
1134	11.7	18.4	13.5	4.8	5.2	3.6	3.10	2.76	2.10	4.20	4.48	4.15	67	61	69	7.37	7.42	7.37	468	410	546	100	88	75	15	22	12
1129	11.5	14.7	12.7	4.4	4.4	3.4	2.80	2.70	1.92	4.20	4.70	3.99	62	64	65	7.42	7.46	7.47	476	460	530	88	100	113	17	20	18
1130	10.8	16.6	14.5	4.4	5.6	2.9	3.54	3.29	2.50	4.00	4.20	3.47	60	60	66	7.40	7.42	7.40	410	388	452	88	88	88	13	18	11
1115	12.6	15.0	12.9	4.4	4.3	3.7	2.17	2.70	2.26	3.77	4.52	3.31	61	62	53	7.40	7.42	7.42	482	482	500	113	88	100	12	9	13
686	12.0	13.9	12.8	5.0	4.3	2.8	2.80	2.50	2.71	3.59	4.10	3.99	57	68	52	7.37	7.37	7.40	480	434	476	113	88	113	14	17	16
1012	12.1	17.1	13.6	4.7	5.2	2.8	2.78	2.59	1.77	3.67	4.27	3.24	57	58	64	7.42	7.40	7.42	452	428	488	88	75	63	12	18	10
1109	12.4	15.4	13.2	5.3	5.6	3.6	2.80	2.30	2.50	3.80	4.80	4.14	58	60	43	7.40	7.40	7.52	450	420	516	113	100	113	11	15	12
1068	12.4	13.6	12.2	5.7	6.2	4.6	–	–	–	–	–	–	57	66	52	7.42	7.40	7.42	484	420	525	125	100	110	12	15	12
614	10.9	13.3	11.9	4.5	4.3	3.1	–	–	–	–	–	–	46	61	57	7.42	7.40	7.42	468	460	500	125	200	113	10	12	9
1047	10.3	16.0	12.3	5.3	4.6	4.6	–	–	–	–	–	–	53	63	58	7.36	7.37	7.42	450	404	510	145	125	125	11	15	11
966	11.8	14.0	12.6	3.4	4.2	3.3	–	–	–	–	–	–	52	64	50	7.38	7.40	7.42	500	452	478	112	138	112	10	13	10
958	11.1	15.7	13.5	3.7	4.0	3.0	–	–	–	–	–	–	58	61	54	7.39	7.41	7.42	500	439	488	114	100	88	12	13	14
1156	10.9	13.3	11.5	4.9	4.6	4.1	2.80	1.87	2.09	4.89	6.18	3.98	56	52	59	7.42	7.47	7.45	429	412	445	75	88	175	12	17	9
1146	10.6	16.3	12.5	5.5	6.6	5.0	2.88	2.59	2.80	3.71	4.22	3.80	57	48	54	7.37	7.45	7.35	464	445	545	75	113	113	18	17	14
1140	9.4	15.4	11.5	4.4	5.6	5.3	2.88	2.48	2.20	4.68	4.14	4.08	63	62	65	7.37	7.37	7.40	368	368	400	63	88	75	22	22	18
Total Av.	11.4	15.2	12.7	4.7	4.9	3.7	2.85	2 58	2.28	4.05	4.56	3.81	59	61	57	7.39	7.41	7.42	459	428	493	102	105	105	14	16	13

*Viosterol 10,000 X, 0.5 c.c. (20 gtt.) administered between eight and fifteen days. Determination made on day drug was discontinued.
†After NaCl, determinations made twelve hours later.

tration of the blood was shown as a slight shift toward the alkaline side. Individual variations of fever may have accounted for an increased P_H in an occasional case. This is especially true after the administration of physiologic saline. This is demonstrated in Case 1,109, Table I. The average P_H for the same group after the intravenous injection of physiologic saline was 7.42. The large quantity of saline used in a short period of time had no deleterious effect on the patients outside of an occasional chill.

These data show that viosterol hypercalcemia is not accompanied by an increase in hydrogen ion concentration. In hypercalcemia produced by viosterol our data do not conform to the opinion quoted by Peters and Van Slyke,[17] that a lower P_H tends to mobilize the calcium in the blood stream. Bernheim[18] states that diffusible calcium increased with a decrease in P_H. The average for 10[19] of the 15 cases (5 additional ones in this report) presented in Table I shows an increase in both diffusible and nondiffusible calcium during the state of hypercalcemia when the P_H was increased. This work demonstrated that viosterol increased the nondiffusible calcium to a much greater extent than the diffusible calcium. At the same time the hydrogen ion concentration was decreased (P_H increased).

Carbon dioxide in this series of 15 cases averaged 59 volumes per cent. During the period of hypercalcemia the average carbon dioxide percentage was 61. Although this percentage is within normal limits it points to a slight increase in carbon dioxide during the state of hypercalcemia. Following the administration of physiologic saline, carbon dioxide averaged 57 volumes per cent. A decrease from 61 to 57 volumes per cent can be accounted for by fever. Collip and Backus[20] were the first to point out that overventilation produces an alkalosis due to washing out of carbon dioxide from the blood. This fact was later borne out by Grant and Goldman.[21] It was further noted by Koehler[22] that acute clinical fevers produce a similar picture, namely, a low CO_2 and a high P_H.

THE EFFECT OF VIOSTEROL ON GLOBULIN AND ALBUMIN

In 10 cases (Table I) the globulin and albumin were analyzed simultaneously with the other determinations. The total protein average was found to be 6.90 gm. per 100 c.c.; globulin was 2.85 gm.; albumin was 4.05 gm. During hypercalcemia the total protein increased to 7.14 gm. per 100 c.c. This increase was due to the elevation of albumin. This is in accord with the finding of other investigators,[23] namely, that proteins permit serum to hold more calcium in solution. When calcium was increased by means of viosterol, it was the albumin fraction which was responsible for retention of the calcium. Globulin decreased during viosterol hypercalcemia. Following the administration of physiologic saline intravenously, the total protein decreased to 6.10 gm. per 100 c.c. Globulin and albumin decreased, the albumin more than the globulin. Further work is required to explain the mechanism by which protein is diminished following administration of intravenous physiologic saline.

THE EFFECT OF VIOSTEROL ON CHLORIDES, GLUCOSE AND UREA NITROGEN

The 15 cases (Table I) showed a normal average of 459 mg. of chlorides preceding the administration of viosterol. During hypercalcemia, the chlorides

averaged 428 mg. per 100 c.c. All but two cases showed a decrease in chlorides. During the intravenous administration of physiologic saline the chlorides were elevated. The average for the 15 cases was 493 mg. per 100 c.c., an increase of 65 mg. per 100 c.c. over the average during the condition of hypercalcemia, and an increase of 44 mg. per 100 c.c. over the normal average.

The average glucose concentration of the blood for the 15 cases was 102 mg. per 100 c.c. After the administration of viosterol the glucose was found to average 105 mg. per 100 c.c. Following the administration of physiologic saline, the average glucose was 105 mg. per 100 c.c. These data present a slight increase in glucose after viosterol. Physiologic saline had no effect on the glucose of the blood.

Although some cases of pulmonary tuberculosis tend to have a slightly elevated urea nitrogen, the average for this group of cases was 14 mg. per 100 c.c. During hypercalcemia, the urea nitrogen showed a tendency to increase in this series of cases. Following the administration of physiologic saline, the urea nitrogen averaged 13 mg. per 100 c.c. Sweany, Weathers and McCluskey[24] observed that in tuberculosis with no serious kidney lesion present, the chlorides and urea nitrogen played a reciprocal rôle. In this series when the chlorides were increased by physiologic saline, the urea nitrogen was slightly decreased.

SUMMARY

1. Viosterol increases the total protein, calcium, and phosphorus in the blood. The albumin fraction of the protein is increased, globulin decreased. The administration of intravenous physiologic saline decreased the total protein, calcium, and phosphorus.

2. The state of hypercalcemia produced by viosterol is not accompanied by an increased hydrogen ion concentration (P_H decreased).

3. The chloride content of the blood is slightly decreased during viosterol hypercalcemia, and the urea nitrogen is very slightly increased.

4. The carbon dioxide content and glucose are not significantly altered by the massive doses of viosterol used to produce hypercalcemia.

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CHEMOTHERAPEUTIC STUDIES WITH SODIUM RICINOLEATE (SORICIN)*

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IN CHEMOTHERAPY and especially of bacterial infections the possibility of producing chemical agents capable of inactivating or destroying toxins and related substances in the blood and other tissues has long been of engaging interest. Of course, the predominating motive of chemotherapeutic research is the production of chemical agents possessing parasiticidal activity in vivo, but almost of equal importance is the possibility of producing compounds of detoxifying activity in vivo even though lacking in demonstrable parasiticidal activity.

This is especially true in many of the bacterial infections not only because exogenous toxins are the chief pathogenic agents in some like diphtheria, tetanus, gangrene, scarlet fever, and erysipelas, but because bacterial products, both toxic and nontoxic, produced in the tissues during infection, would appear capable of retarding or preventing phagocytosis and preventing the activity of humoral antibodies. In the case of some bacterial infections these agents like the aggressins do not appear to be primarily toxic but are, nevertheless, pathogenic in this sense of retarding or preventing phagocytosis. In pneumococcus pneumonia for example, it would appear that the nontoxic and nonantigenic soluble substance "S" discovered by Avery and his coworkers has the faculty of neutralizing the antibodies produced by the patient and enhancing the virulence of the organisms by preventing their phagocytosis.

At the present time our main hope of combating the toxins and these nontoxic agents is by means of specific immune sera but the possibility of discovering chemical agents capable of inactivating or completely destroying them in vivo is of fundamental importance.

From this standpoint we have been greatly interested in sodium ricinoleate, that is, in the possibility of this compound destroying these toxic and nontoxic bacterial agents in vivo and especially since the investigations of Larson and his colleagues,[1] Sommer and Sommer,[2] Netter, et al.,[3] Dorst and Morris[4] and others, the literature being briefly summarized by Harris and Bunker,[5] have indicated that sodium ricinoleate is capable of neutralizing various bacterial toxins, phytotoxins and cobra venom in the test tube and possibly in the living animal in the case of some of them with special reference to streptococci and pneumococci.

Furthermore in chemotherapeutic research there is always the possibility of compounds inert in the test tube undergoing transformation in the living

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Received for publication, September 14, 1933.

animal into new and more active antiparasiticidal agents. For this reason test tube bactericidal activity in vitro may be without curative activity in the living animal or, being extremely low in parasiticidal activity in the test tube, undergo transformation in the blood or tissues into compounds of extremely high curative activity as in the case of arsphenamine, neoarsphenamine, and other trivalent organic arsenical compounds. For these reasons test tube determinations of bactericidal activity are of most value in the case of those compounds to be used in treatment by local application, whereas in the case of those designed for parenteral administration actual tests with experimental infections in living animals are required. For example, we think it is extremely fortunate that Ehrlich and his colleagues did not have cultures of trypanosomes and *Spirocheta pallida* available in 1907, for they may have committed the error of testing their new arsenical compounds, including salvarsan, in the test tube only and finding them so low in parasiticidal activity may have neglected their administration to infected animals and thereby missed or delayed making their great discoveries. Therefore, even though we knew sodium ricinoleate to be of very low bactericidal activity in the test tube, we have thought it worth while to administer it in the treatment of various experimental infections and especially since other investigators have reported results lending some encouragement to these efforts.

Furthermore the studies of Larson and his colleagues and others have indicated the possibility of sodium ricinoleate transforming some of the bacterial toxins into toxoids or other compounds of greatly reduced toxicity in the test tube but retaining their antigenic properties, and thereby of interest from the standpoint of vaccination against diphtheria, scarlet fever, erysipelas, etc. But this is a study in immunity rather than in chemotherapy and has not engaged our particular interest in these investigations, the results of which are briefly summarized in this communication.

TOXICITY OF SODIUM RICINOLEATE

The compound employed in this study* was prepared by Rider[6] with a high degree of purity and its toxicity determined by intravenous, intraperitoneal, intramuscular and subthecal administration preliminary to trial in the treatment of several experimental bacterial septicemias and toxinemias, trypanosomiasis, syphilis, and poliomyelitis of the lower animals.

(a) *By Intravenous Injection.*—When 2 per cent solutions were given rats intravenously with a gravity apparatus at the rate of 1 c.c. in two minutes, as employed in determining the toxicity of arsphenamine and neoarsphenamine,[7] the maximum tolerated dose permitting animals to survive ten days or longer was approximately 0.035 gm. per kilogram weight (Table I) while 0.050 gm. per kilogram produced death immediately or within one or two days (minimal lethal dose). It is, therefore, about 4 times as toxic as alkalinized solutions of arsphenamine by this route of administration. When the injections were given more rapidly and especially with more concentrated solutions, the tolerance was greatly reduced, showing that for this route of administration it is necessary to use more dilute solutions slowly injected.

*Kindly furnished by the William S. Merrell Company of Cincinnati, Ohio.

Rabbits have borne 10 daily doses of 0.010 gm. per kilogram and 0.005 gm. per kilogram in as many as 30 consecutive daily doses without untoward effects, except the production of sclerosis and thrombosis of the marginal ear veins and microscopic examination of the lungs, heart, liver, and kidneys have shown practically no histologic evidences of injury. However, the heart and lungs of rabbits and rats succumbing immediately after the injection of lethal doses have shown large emboli of hemolyzed erythrocytes, the whole histologic picture bearing a striking resemblance to those changes described by Kolmer and Lucke[8] following the intravenous injection of acid solutions of arsphenamine, producing intravascular hemolysis and agglutination.

TABLE I

TOXICITY OF SODIUM RICINOLEATE

ANIMAL	ROUTE OF ADMINISTRATION	SOLUTION	MINIMAL LETHAL DOSE PER KILO	MAXIMUM TOLERATED DOSE PER KILO
Rat	Intraven.	2.0%	0.050 gm.	0.035 gm.*
Rat	Intramus.	10.0%	–	More than 1.0 gm.
Rat	Intraperit.	4.0%	0.5 gm.	0.3 gm.
Mice	Intraperit.	4.0%	0.5 gm.	0.25 gm.
Guinea pig	Intraperit.	4.0%	–	0.16 gm.
Guinea pig	Intraspinal	0.1%	–	0.004 gm.

*Rabbits have tolerated 10 daily doses of 0.010 gm. and 30 daily doses of 0.005 gm. per kilogram without untoward effects.

TABLE II

HEMOLYTIC ACTIVITY OF SODIUM RICINOLEATE IN WATER-BATH AT 37° C.

WASHED ERYTHROCYTES				WHOLE DEFIBRINATED BLOOD			
SODIUM RICINOLEATE	5% CORPUSCLES	FINAL DIL.	HEMOLYSIS	SODIUM RICINOLEATE	BLOOD	FINAL DIL.	HEMOLYSIS
1 c.c. 1: 25	1 c.c.	1: 50	At once	1 c.c. 1: 25	1 c.c.	1: 50	At once
1 c.c. 1: 50	1 c.c.	1: 100	At once	1 c.c. 1: 50	1 c.c.	1: 100	At once
1 c.c. 1: 100	1 c.c.	1: 200	Few seconds	1 c.c. 1: 100	1 c.c.	1: 200	Few seconds
1 c.c. 1: 200	1 c.c.	1: 400	Few seconds	1 c.c. 1: 200	1 c.c.	1: 400	Few seconds
1 c.c. 1: 400	1 c.c.	1: 800	15 seconds	1 c.c. 1: 400	1 c.c.	1: 800	About minute
1 c.c. 1: 800	1 c.c.	1:1,600	About minute	1 c.c. 1: 800	1 c.c.	1:1,600	20 minutes
1 c.c. 1:1,600	1 c.c.	1:3,200	About minute	1 c.c. 1:1,600	1 c.c.	1:3,200	Incomplete
1 c.c. 1:3,200	1 c.c.	1:6,400	30 minutes	1 c.c. 1:3,200	1 c.c.	1:6,400	Incomplete

(b) *Hemolytic and Agglutinating Activity.*—Indeed 1 c.c. of 1:3200 solution of sodium ricinoleate in saline solution is capable of completely hemolyzing 1 c.c. of 5 per cent suspension of washed rabbit corpuscles (final dilution 1:6400) in about thirty minutes at 37° C. (Table II). Even in the presence of plasma, the hemolytic activity of the compound is quite high, since 1 c.c. of 1:800 dilution has produced complete hemolysis of 1 c.c. of defibrinated rabbit blood (final dilution 1:1600) within twenty minutes in a water-bath at 37° C.

Curiously, however, sodium ricinoleate does not produce any marked degree of agglutination of either washed or unwashed erythrocytes in the test tube, and it would appear that intravascular agglutination plays little or no part in toxicity by intravenous injection, although it may be that the emboli found in the heart and vessels of the lungs as well as to some extent in the kidneys are

due to thromboplastic stromas and other débris of hemolysis as suggested by Gerwe[9] and responsible for the respiratory and cardiac failure resulting in death following intravenous injections of lethal amounts.

(c) *By Intramuscular Injection.*—By intramuscular injection, however, the hemolytic and indirect thromboplastic effects of sodium ricinoleate are largely removed and consequently the toxicity is greatly reduced, since rats have withstood doses larger than 1.0 gm. per kilogram of weight (Table I). Not infrequently, however, abscesses have resulted at the sites of injection showing that the compound produces considerable inflammation with liquefaction necrosis by this route of administration, the pus being frequently of a brownish color due to local hemolysis.

(d) *By Intraperitoneal Injection.*—The same has been found true after intraperitoneal injections in rats, mice, and guinea pigs in that upon autopsy extensive inflammatory changes of the parietal and visceral peritoneum was sometimes observed along with light reddish-brown exudates of a soapy nature. Indeed rats and mice usually succumbed within twenty-four hours to doses of 0.5 gm. per kilogram (minimal lethal dose) although single doses as high as 0.25 to 0.3 gm. per kilogram (Table I) were borne indefinitely (maximum tolerated dose). With guinea pigs, however, the maximum tolerated dose was somewhat less being about 0.16 gm. per kilogram which corresponds quite closely to the dose of 0.12 to 0.27 gm. per kilo observed by Gerwe.[9]

(e) *By Intracisternal Injection.*—Since we proposed using sodium ricinoleate in the treatment of tetanus of guinea pigs by intraspinal injection it was necessary to determine its toxicity by this route of administration, and in guinea pigs it was found that the maximum tolerated dose by intracisternal injection was approximately 0.004 gm. per kilo or about one-tenth of the maximum tolerated dose by intravenous injection (Table I).

THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN

Along with a large number of other compounds[5] sodium ricinoleate is capable of inactivating diphtheria toxin in the test tube. As shown in Table III as an example of the results of several experiments, an amount as small as 0.0075 gm. dissolved in 1 c.c. of saline solution and added to 6 M. L. D. of diphtheria toxin in 1 c.c. is capable of completely inactivating the latter during an exposure of one hour at room temperature as determined by the subcutaneous injection of the 2 c.c. mixtures into young guinea pigs (8 to 10 ounces). When, however, an L+ dose of toxin is employed, amounts of sodium ricinoleate ranging from 0.003 to 0.030 gm. are unable to completely detoxify this large amount of toxin in the test tube.

Whether or not the detoxified mixtures are capable of engendering immunity, that is, of retaining antigenic activity, has not been definitely determined. In one experiment we partially detoxified 5 L+ doses with 0.1 gm. of sodium ricinoleate dissolved in 50 c.c. of saline and allowed to stand in an incubator for one hour and kept in a refrigerator. Twelve guinea pigs were subsequently given 1 c.c. subcutaneously every five days. Of these 8 died after 5 to 8 injections due to incomplete detoxification of the toxin. Four survived

and three weeks after the last dose were given a subcutaneous injection of 3 M. L. D.'s of toxin sufficient for producing fatal intoxication of 4 controls on the third day after injection; the immunized pigs succumbed in from six to eight days, so that it would appear that but little immunity had been engendered and suggesting that sodium ricinoleate may de-antigenize a portion of diphtheria toxin in vitro.

TABLE III

THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN IN VITRO

DIPHTHERIA TOXIN (1 C.C.)	SODIUM RICINOLEATE (GM.)	RESULTS IN DAYS										
		1	2	3	4	5	6	7	8	9	10	12
1 L + dose	0.003	–*	D									
1 L + dose	0.003	–	D									
1 L + dose	0.006	–	–	D								
1 L + dose	0.006	–	–	–	D							
1 L + dose	0.0075	–	D									
1 L + dose	0.0075	–	–	–	D							
1 L + dose	0.010	–	D									
1 L + dose	0.010	–	D									
1 L + dose	0.015	–	–	D								
1 L + dose	0.015	–	–	–	D							
1 L + dose	0.030	–	–	D								
1 L + dose	0.030	–	–	–	–	D						
6 M. L. D.	0.003	–	–	–	–	D						
6 M. L. D.	0.006	–	–	–	–	–	–	–	D			
6 M. L. D.	0.0075	–	–	–	–	–	–	–	–	–	–	–
6 M. L. D.	0.010	–	–	–	–	–	–	–	–	–	–	–
6 M. L. D.	0.015	–	–	–	–	–	–	–	–	–	–	–
6 M. L. D.	0.030	–	–	–	–	–	–	–	–	–	–	–
Control (1 L+)	0	–	D									
Control (1 L+)	0	–	D									
Control (6 M.L.D.)	0	–	D									
Control (6 M.L.D.)	0	–	D									

* – = survived: D = died.

However, of much more interest from the standpoint of chemotherapy is the question of whether or not sodium ricinoleate is capable of destroying diphtheria toxin in the living guinea pig.

For this purpose animals were given subcutaneous injections of 6 M. L. D.'s of toxin subcutaneously and sodium ricinoleate injected intravenously twenty-four hours later in doses ranging from 0.003 to 0.030 gm. per kilo. In other experiments the sodium ricinoleate was given intramuscularly in doses ranging from 0.1 to 0.5 gm. per kilogram, immediately after the subcutaneous injection of the toxin.

As shown in Table IV the untreated controls succumbed in forty-eight hours after the injection of toxin, as the dose was rather large but purposely selected in order to leave no doubt about the results. But unfortunately the sodium ricinoleate in the doses administered was unsuccessful in every instance in combating the toxemia, although the larger doses sometimes prolonged the lives of the animals by one or two days.

Duplicate tests embracing the intraperitoneal injection of sodium ricinoleate in doses ranging from 0.010 to 0.050 gm. per kilogram four hours after

TABLE IV

THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN IN VIVO

DIPHTHERIA TOXIN* (1 C.C.)	SODIUM RICINOLEATE		RESULTS IN DAYS					
	DOSE PER KILO	ADMINISTRATION	1	2	3	4	5	6
6 M. L. D.	0.003	Intravenously**	–‡	D				
6 M. L. D.	0.006	Intravenously**	–	D				
6 M. L. D.	0.0075	Intravenously**	–	D				
6 M. L. D.	0.010	Intravenously**	–	–	D			
6 M. L. D.	0.015	Intravenously**	–	D				
6 M. L. D.	0.030	Intravenously**	–	–	–	D		
6 M. L. D.	0.1	Intramuscularly†	–	D				
6 M. L. D.	0.15	Intramuscularly†	–	D				
6 M. L. D.	0.2	Intramuscularly†	–	D				
6 M. L. D.	0.3	Intramuscularly†	–	–	D			
6 M. L. D.	0.4	Intramuscularly†	–	D				
6 M. L. D.	0.5	Intramuscularly†	–	–	D			
3 M. L. D.	0.010	Intraperitoneal§	–	–	–	D		
3 M. L. D.	0.020	Intraperitoneal§	–	–	–	–	D	
3 M. L. D.	0.040	Intraperitoneal§	–	–	–	D		
3 M. L. D.	0.050	Intraperitoneal§	–	–	–	D		
Control (6 M. L. D.)	0	0	–	D				
Control (6 M. L. D.)	0	0	–	D				
Control (6 M. L. D.)	0	0	–	D				
Control (6 M. L. D.)	0	0	–	D				
Control (3 M. L. D.)	0	0	–	–	–	D		
Control (3 M. L. D.)	0	0	–	–	–	D		
Control (3 M. L. D.)	0	0	–	–	–	D		

*By subcutaneous injection.
†Immediately after injection of toxin.
**Twenty-four hours after injection of toxin.
‡ – = survived; D = died.
§Given four hours after subcutaneous injection of toxin and repeated daily for 3 more doses.

the subcutaneous injection of 3 M. L. D.'s of toxin and repeated daily for 3 additional doses gave similar negative results, and as the experiments were repeated several times, it would appear that the compound is incapable of appreciably detoxifying diphtheria toxin in the living animal.

THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN

Similar results were observed with tetanus toxin except to state that in the test tube, sodium ricinoleate appears capable of detoxifying this toxin to a somewhat greater degree than diphtheria toxin.

For example an L+ dose of tetanus toxin in 1 c.c. of saline solution is apparently completely detoxified by 0.006 gm. of sodium ricinoleate dissolved in 1 c.c. of saline solution in an hour at room temperature as determined by injecting the mixtures subcutaneously into young guinea pigs (Table V).

Even a dose as small as 0.003 gm. detoxified this amount of toxin sufficiently to permit the animals to survive several days after the death of controls injected with the toxin alone.

Unfortunately, however, this inactivated toxin does not appear to possess antigenic value if one may judge from the results of a single experiment in which 5 L+ doses of toxin were added to 0.1 gm. of sodium ricinoleate dissolved in 50 c.c. of saline and incubated for an hour at 37° C. followed by

preservation in a refrigerator. Twelve guinea pigs were given 1 c.c. subcutaneously every five days for 10 doses. One animal succumbed before the series of injections were completed. Three weeks after the last dose the remaining

TABLE V

THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN IN VITRO

TETANUS TOXIN (1 C.C.)	SODIUM RICINOLEATE (GM.)	RESULTS IN DAYS										
		1	2	3	4	5	6	7	8	9	10	12
1 L + dose	0.003	–*	–	–	–	–	–	D				
1 L + dose	0.003	–	–	–	–	D						
1 L + dose	0.006	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.006	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.0075	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.0075	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.01	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.01	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.015	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.015	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.030	–	–	–	–	–	–	–	–	–	–	–
1 L + dose	0.030	–	–	–	–	–	–	–	–	–	–	–
Control	0	–	D									
Control	0	–	D									
Control	0	–	D									
Control	0	–	D									

* – = survived: D = died.

11 were given a subcutaneous injection of one-one-hundred-fiftieth of an L+ dose of toxin sufficient to cause the death of 4 controls on the third day. All of the immunized animals died in from five to eleven days, indicating that some immunity had been produced, but likewise, suggesting that sodium ricinoleate tends to de-antigenize a portion of this toxin as was probably also true of diphtheria toxin previously described.

But it would appear that sodium ricinoleate is capable of detoxifying tetanus toxin to some slight extent in the living animal.

Controls injected subcutaneously with one L+ dose of toxin died of tetanus intoxication in about forty-eight hours, whereas when 0.5 gm. of sodium ricinoleate was given intramuscularly, immediately after the toxin, some animals lived for one or two days longer (Table VI).

Controls injected subcutaneously with one-one-hundredth of the L+ dose died of tetanus in about three or four days, whereas the intramuscular injection of sodium ricinoleate in doses of 0.1 to 0.5 gm. per kilo immediately after the injection of toxin, prolonged the lives of some animals by one or two days.

Intravenous injections of 0.003 to 0.030 gm. per kilo twenty-four hours after the injection of toxin had no appreciable effect and the same was generally true of intraperitoneal injections. In the latter experiments guinea pigs were given subcutaneous injections of one-one-hundredth of the L+ dose of toxin sufficient to produce fatal tetanus intoxication in from three to four days. Four hours after the injection of toxin sodium ricinoleate was given intraperitoneally in doses varying from 0.010 to 0.040 gm. per kilo and repeated daily for 4 addi-

tional doses. All of the animals, however, developed tetanus and died in from four to six days, living, therefore, but a few days beyond the controls (Table VI).

Additional guinea pigs were given subcutaneous injections of one-one-hundredth of the L+ dose of toxin and four days later when symptoms of tetanus first appeared were given 0.003 gm. of sodium ricinoleate intraspinally. This dose was repeated daily for 2 additional doses but all of the animals died in five or six days while the untreated controls succumbed in three or four days (Table VI).

TABLE VI

THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN IN VIVO

TETANUS TOXIN* (1 C.C.)	SODIUM RICINOLEATE		RESULTS IN DAYS					
	DOSE PER KILO	ADMINISTRATION	1	2	3	4	5	6
1 L + dose	0.1	Intramuscular**	–‡	D				
1 L + dose	0.1	Intramuscular**	–	D				
1 L + dose	0.25	Intramuscular**	–	D				
1 L + dose	0.25	Intramuscular**	–	D				
1 L + dose	0.5	Intramuscular**	–	–	D			
1 L + dose	0.5	Intramuscular**	–	–	–	D		
1/100 of L + dose	0.003	Intravenously†	–	–	–	D		
1/100 of L + dose	0.006	Intravenously†	–	–	–	D		
1/100 of L + dose	0.075	Intravenously†	–	–	–	–	D	
1/100 of L + dose	0.010	Intravenously†	–	–	–	D		
1/100 of L + dose	0.015	Intravenously†	–	–	–	D		
1/100 of L + dose	0.030	Intravenously†	–	–	D			
1/100 of L + dose	0.010	Intraperitoneally	–	–	–	D		
1/100 of L + dose	0.015	Intraperitoneally	–	–	–	D		
1/100 of L + dose	0.020	Intraperitoneally	–	–	–	–	D	
1/100 of L + dose	0.030	Intraperitoneally	–	–	–	–	D	
1/100 of L + dose	0.040	Intraperitoneally	–	–	–	–	–	D
1/100 of L + dose	0.1	Intramuscularly	–	–	–	–		
1/100 of L + dose	0.2	Intramuscularly	–	–	–	–		
1/100 of L + dose	0.3	Intramuscularly	–	–	–	D		
1/100 of L + dose	0.4	Intramuscularly	–	–	–	D		
1/100 of L + dose	0.5	Intramuscularly	–	–	–	–	–	D
1/100 of L + dose	0.003	Intraspinally	–	–	D			
1/100 of L + dose	0.003	Intraspinally	–	–	–	–	D	
1/100 of L + dose	0.003	Intraspinally	–	–	–	–	–	D
1/100 of L + dose	0.003	Intraspinally	–	–	–	–	–	D
Control (1 L +)	0	0	–	D				
Control (1 L +)	0	0	–	D				
Control (1/100 L +)	0	0	–	–	D			
Control (1/100 L +)	0	0	–	–	–	D		
Control (1/100 L +)	0	0	–	–	D			
Control (1/100 L +)	0	0	–	–	D			

*By subcutaneous injection. **Immediately after injection of toxin.
†Twenty-four hours after injection of toxin. ‡ – = survived; D = died.

These experiments repeated several times have shown, therefore, that sodium ricinoleate may have a slight degree of detoxifying activity for tetanus toxin in vivo; the doses of toxin were relatively large in order to produce death from tetanus of all of the controls which may have masked more evidence of detoxification in these experiments.

THE BACTERICIDAL ACTIVITY OF SODIUM RICINOLEATE

Sodium ricinoleate has a low bactericidal activity in the test tube.

When tested according to the Reddish method against *Staphylococcus pyogenes aureus,* it was found that whereas a 20 per cent solution was completely

bactericidal for this organism in an exposure of five minutes, yet 10 and 5 per cent solutions were not completely bactericidal in exposures as long as one hour. These results correspond closely to those of Gerwe[9] who also found the phenol coefficient for *B. typhosus* very low.

By the bacteriostatic method of Kolmer,[10] however, we have found that 1:4,000 concentrations of sodium ricinoleate inhibited the growth of *Staphylococcus aureus* in broth medium while 1:600 dilutions were bactericidal. With *Streptococcus hemolyticus* dilutions of 1:60,000 were bacteriostatic and 1:40,000 bactericidal; with Pneumococcus (Type I) dilutions up to 1:80,000 were bacteriostatic and 1:60,000 bactericidal.

SODIUM RICINOLEATE IN THE TREATMENT OF VARIOUS EXPERIMENTAL INFECTIONS

In Streptococcus Cellulitis and Peritonitis of Mice.—White mice were given intraperitoneal injections of 0.6 c.c. of twenty-four-hour broth cultures of *Streptococcus hemolyticus* and four hours later intraperitoneal injections of sodium ricinoleate in doses varying from 0.050 to 0.250 gm. per kilogram of weight.

The culture was of such virulence that the 8 untreated controls died of the resulting peritonitis and septicemia in about forty-eight hours, all animals showing positive heart blood cultures at necropsy.

All of 20 mice receiving the compound died, however, in the same period of time so that single doses of sodium ricinoleate of the amounts employed were without appreciable effect. All of these mice, likewise, showed positive heart blood cultures.

Fourteen additional mice were infected with the same dose of culture by injection under the skin of the thorax. Four untreated controls died in from three to five days of the resulting cellulitis and with positive heart blood cultures obtained at autopsy.

Ten mice were given intraperitoneal injections of single doses of sodium ricinoleate, likewise varying from 0.050 to 0.250 gm. per kilogram of weight, but all of these also perished in about the same period of time as the untreated controls from the local abscesses associated with the septicemia (positive heart blood cultures).

From these experiments it appeared that sodium ricinoleate by intraperitoneal injection was without appreciable curative effects in streptococcus cellulitis and peritonitis of mice. It is true, however, that the infections were very severe but unfortunately this is a difficulty in the chemotherapy of experimental streptococcus infections of mice in that smaller doses of the organism permits some untreated controls to live indefinitely and therefore making it difficult to evaluate the results of treatment. On the other hand the rapidly fatal experimental infections have no parallel in streptococcus infections of the human being and may readily enough mask some degree of curative activity on the part of the compound under study.

In Pneumococcus Peritonitis and Septicemia of Mice.—Similar negative results were observed in the treatment of mice infected with a highly virulent culture of Type I pneumococcus.

Eight mice injected intraperitoneally with 0.3 c.c. of a 1:40,000 dilution of twenty-four-hour broth culture died in about forty-eight hours with positive heart blood cultures (septicemia). Twenty additional mice infected at the same time were given single doses of sodium ricinoleate intraperitoneally four hours later in doses varying from 0.006 to 0.050 gm. per kilogram of weight. All of these, likewise, succumbed in about the same time as the untreated controls, so that in this single experiment there was no evidence of curative activity on the part of the compound administered by intraperitoneal injection.

In Tuberculosis of Guinea Pigs.—A series of 24 guinea pigs weighing from 280 to 400 gm. were given subcutaneous injections in the neighborhood of the inguinal glands of 0.005 gm. of human tubercle bacilli. Four untreated controls died of the resulting infection in from twelve to eighteen days.

One week after infection 20 of the animals were given intraperitoneal injections of sodium ricinoleate in doses varying from 0.030 to 0.100 gm. per kilo of weight and the injections repeated every three days. Two of the animals receiving the 0.100 gm. dose died after three injections from the toxicity of the drug and before tuberculous lesions had well developed. From 2 to 8 injections of sodium ricinoleate were given the 18 remaining animals, but all died of widespread tuberculous infection in from eight to twenty days, so that the compound was without appreciable curative effects. Indeed a number of the animals receiving the larger doses of sodium ricinoleate died a few days earlier than the untreated controls and suggesting that their resistance to tuberculous infection had been reduced by the compound.

In Syphilis of Rabbits.—Eight rabbits were given intratesticular inoculations with our Nichols-Hough strain of *Spirocheta pallida.* One month later two untreated controls developed acute bilateral orchitis with numerous spirochetes in dark-field examinations.

One week after testicular inoculation 6 of the rabbits were given 0.003 gm. of sodium ricinoleate per kilogram of weight by intravenous injection, and the dose was repeated daily for a total of 8 injections.

All of these animals likewise developed acute testicular syphilis with positive dark-field examinations in about a month, so that the compound was without any demonstrable curative effects in this single experiment.

In Trypanosomiasis of Rats.—White rats weighing from 110 to 300 gm. were given intraperitoneal injections of approximately 500,000 *Tryp. equiperdum.* Four untreated controls died of the resulting infection in from three to four days and showed enormous numbers of the trypanosomes in the tail blood.

Twenty-four hours after infection and before parasites appeared in the peripheral blood, 20 of the rats were given single intravenous injections of sodium ricinoleate in doses varying from 0.005 to 0.025 gm. per kilogram of weight. All of these likewise succumbed to trypanosomiasis in from three to four days and showed enormous numbers of parasites in the peripheral blood, so that the compound was without appreciable trypanocidal activity in this single experiment.

In Acute Poliomyelitis of Monkeys.—Three monkeys were inoculated intracerebrally with an emulsion of monkey poliomyelitic spinal cord kindly fur-

nished by the Rockefeller Institute. The first clinical evidences of poliomyelitis was apparent in from seven to nine days, and an untreated control died of paralyis twenty-one days after inoculation.

The two remaining animals were given sodium ricinoleate in dose of 0.005 gm. per kilogram on the seventh day after inoculation when signs of infection were first apparent and the dose repeated daily for a total of five injections. Both animals died in from eighteen to twenty-two days from progressive paralysis, so that sodium ricinoleate was without demonstrable curative effects in these two animals, a more complete description of the experiment being given in a separate communication.[11]

SUMMARY

1. Sodium ricinoleate (soricin) is compound of particular interest from the chemotherapeutic standpoint because of the possibility of inactivating toxins and other bacterial agents in the blood.

2. Its toxicity has been determined by intravenous, intraperitoneal, intramuscular and subthecal injection in the lower animals.

3. It is highly hemolytic and upon intravenous injection may produce intravascular hemolysis with embolism. The maximum tolerated dose by this route of administration has been found to be approximately 0.035 gm. per kilo.

4. By intramuscular injection it may produce local irritation with liquefaction necrosis when large amounts are injected. However it is well borne systematically by this route of administration as the maximum tolerated dose has been found to be more than 1 gm. per kilo.

5. It may also produce some irritation upon intraperitoneal injection but is well borne by this route, since the maximum tolerated dose is as high as 0.16 to 0.3 gm. per kilo.

6. By intracisternal injection the maximum tolerated dose has been found to be approximately 0.004 gm. per kilo.

7. Sodium ricinoleate is capable of inactivating or destroying diphtheria and tetanus toxins in the test tube and is especially destructive for the latter, although it would appear that the resulting toxoids are of low antigenic activity.

8. Sodium ricinoleate is but feebly antitoxic for the toxins of diphtheria and tetanus bacilli in guinea pigs by various routes of administration.

9. Sodium ricinoleate is of very low bactericidal activity in the test tube and has been found without demonstrable curative activity in severe streptococcal, pneumococcal, and tuberculous infections of the lower animals as well as in experimental trypanosomiasis of rats, syphilis of rabbits, and acute anterior poliomyelitis of monkeys.

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THE SPECIFIC GRAVITY OF THE BLOOD IN HUMAN CANCER*

Four Hundred Observations, with a Note on its Clinical Significance

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Normal Values.—The normal specific gravity of the whole blood has been fairly consistently found to be about 1.055 for males and 1.053 for females (Jones,[1] Schmaltz,[2] Lyonnet,[3] Leake, Kohl, and Stebbins,[4] Polowe[5]). A diurnal variation was noted by Jones, Leake et al., and Polowe, the specific gravity of the whole blood being about 0.002 higher in the morning than in the afternoon. For the purposes of this study specific gravity values below 1.050 were considered as representing a state of anemia, while values above 1.060 were considered as representing a higher than normal density of the whole blood.

Literature.—No large series of observations on the specific gravity of the whole blood in human cancer has been made in recent years. Lloyd Jones, in 1887, reported a series of 15 cases. His lowest blood density was 1.031 obtained in a case of carcinoma of the uterus. But even in this small series he called attention to the fact that normal specific gravity values may be found in advanced cases of cancer. Schmaltz, in 1891, reported one case of carcinoma of the stomach in which a specific gravity of the blood of 1.039 was found. Polowe,[6] in 1929, reported his findings in 23 cases of proved carcinoma. He stated at that time that, "the more severe anemias occurred in carcinoma of hidden or not readily accessible areas (gastrointestinal tract, peritoneum, pancreas, urinary bladder). Carcinoma of other parts of the body did not seem to alter the normal blood density, even though most of the cases were quite advanced."

*Received for publication, September 20, 1933.
This work has been aided by a grant from the Committee on Scientific Research of the American Medical Association.

The Clinical Significance of the Specific Gravity of the Blood in Human Cancer.—(a) Any discussion of the specific gravity of the blood presupposes the idea that a change in the quality of the blood is at once manifested by a change in its specific gravity. Such a change may be brought about by an alteration in the number of erythrocytes, leucocytes, platelets; by alteration of the hemoglobin content, fibrinogen content, ash content; by alteration of the lipoid values; and by shifting water balances. In anemia associated with cancer* (as in the anemia associated with other clinical entities) no one factor alone may be incriminated as the sole agent which so materially affects the weight of the blood.

Lucas,[7] in a very recent publication on the diagnosis and treatment of the anemias of cancer, states that if one searches diligently enough the cause of such anemias can usually be found and treated. He states further: "Anemia obviously may result from an increased destruction or loss of blood, from a decreased blood formation, or combination of these two. Decreased blood formation frequently is preceded by a chronic blood loss or destruction. Chronic and acute hemorrhage is usually not difficult to find so that we are chiefly concerned with decreased blood formation due to nutritional deficiency or to the inability of the patient to assimilate food properly." Lucas mentions the following known values to aid in determining the hematopoietic needs of the patient:

1. Red blood cell count
2. The percentage hemoglobin
3. The mean corpuscular volume
4. The color index
5. The free HCl of the gastric contents
6. The saturation index

I have quoted Lucas rather fully because I feel that for the average practitioner the specific gracity of the whole blood offers a rapid orientation of the status of the blood at any given moment and includes at least three (1, 2, and 3 above) of the needed values, and possibly five (the saturation index and, in cases of secondary anemia, the color index). Interpolations of specific gravity values into the above values may be made roughly by experience with the method or quite accurately by the use of Guthrie's comparison chart.[8] One should not forget the use of one's clinical judgment in evaluating any of these findings, including the specific gravity of the blood.

Factors that tend to increase the blood density should also be kept in mind when evaluating the blood status in any given case. An absolute increase in blood density may be brought about by an increase in the production of any of its various constituents. Examples of this may be found in polycythemia vera wherein the erythrocytes are markedly increased in number, though one should note that in this condition the percentage hemoglobin is relatively low; in hyperparathyroidism the increased calcium content of the blood increases the blood density. A relative increase in blood density may be brought about by dehydration due either to lack of fluid intake, lack of fluid intake plus anuria (Popel's[9] work on dogs), or to loss of fluids by excessive diaphoresis, emesis,

*The term "anemia of cancer" should perhaps be avoided. The term "anemia associated with cancer" is suggested to emphasize the clinical fact that the anemia associated with cancer is almost never due to the cancer process itself.

diarrhea, or combinations of these. A relative increase in blood density may also be brought about by venestasis (Copeman's[10] work on rabbits) due to cardio-respiratory diseases or due to actual mechanical obstruction of the venous return by masses in the axilla, neck, thorax, and abdomen as may sometimes be seen in advanced cases of neoplastic disease. In the clinical evaluation of the blood status of the dehydrated carcinomatous patient I have found that the relatively high hemoglobin percentages found are often belied by low specific gravity values of the whole blood, the latter values being more in keeping with the clinical appearance of the patient. Examples of this may be found in cases of obstructive lesions of the stomach wherein vomiting (with due respect paid to starvation effects) causes not only a loss of water but of minerals, such as chlorides, as well. This produces a mineral imbalance between the blood stream and the tissue fluids which is more truly, though not ideally, reflected by the specific gravity of the whole blood than by the estimation of the now concentrated erythrocyte and hemoglobin values. An emotional anhydremia in needle-shy patients has been reported by Barbour and Hamilton.[11]

(b) The following is a study of the blood status associated with human cancer in terms of the specific gravity of the whole blood. The falling drop technic of Barbour and Hamilton[12] was used. The blood was obtained from the capillary bed at the finger tip. In lesions of the breast, axillae, and upper extremities, where only one side was involved, the finger on the sound side was selected to obviate as much as possible the venestatic effect of mechanical pressure.

Single observations were made on 400 cases of proved cancer (Table I) and, as a control on 50 cases of proved benign neoplasms and noncancerous lesions (Table II). Table III represents a further control group of 50 cases, in summary form, of average run, nonneoplastic conditions as found in a general hospital, the details of which were published elsewhere.[5, 6] Tables IV, V, and VI, respectively, represent the severe, the moderately severe, and the mild cases of the anemias associated with cancer as found in this study. The interpolations of the specific gravity values, as shown in Tables IV, V, and VI, into

TABLE I

THE SPECIFIC GRAVITY OF THE WHOLE BLOOD
(COMPOSITE SUMMARY OF FINDINGS IN 400 CASES OF HUMAN CANCER)

CLINIC	NO. OBS.	SP. GR. BELOW 1.050	PER CENT ANEMIC	SP. GR. 1.060 AND OVER	PER CENT WITH HIGH SP. GR.
A. Breast	100	3	3	13	13
B. Epitheliomas	23	1	4	5	22
C. Sarcomas, neur.	50	6	12	9	18
D. Rectal	100	20	20	1	1
E. Gynecologic	50	8	16	3	6
F. Oral	26	3	12	1	4
G. Gastric	20	12	60	0	0
H. Sarcomas, osteo.	15	3	20	0	0
I. Urologic	16	8	50	0	0
	400	64	16	32	8
Corrected	900	197	22	64	7

hemoglobin content, red blood cell counts, and sediment volume percentage, were made from the comparison chart of Guthrie.[8] Notes (summarized in Table VII) were made as to the grades of malignancy and as to the pathologists' estimate

TABLE II

THE SPECIFIC GRAVITY OF THE WHOLE BLOOD
(COMPOSITE SUMMARY OF FINDINGS IN 50 CASES OF NONCANCEROUS LESIONS)

CLINIC	NO. OBS.	SP. GR. BELOW 1.050	PER CENT ANEMIC	SP. GR. 1.060 AND OVER	PER CENT WITH HIGH SP. GR.
B. Epitheliomas	13	1	8	0	0
E. Gynecologic	18	2	11	1	5
G. Gastric	14	4	30	0	0
H. Bone	2	0	0	1	50
I. Urologic	3	3	100	0	0
	50	10	20	2	4
Corrected	500	149	30	55	11

TABLE III

THE SPECIFIC GRAVITY OF THE WHOLE BLOOD*
(FINDINGS IN 50 MISCELLANEOUS CASES AS FOUND IN A GENERAL HOSPITAL)

WARDS	NO. OBS.	SP. GR. BELOW 1.050	PER CENT ANEMIC	SP. GR. 1.060 AND OVER	PER CENT WITH HIGH SP. GR.
Various	50	26	52	1†	2

*From the Barnert Memorial Hospital, Paterson, N. J. [5, 6]
†Diagnosis: Bronchial asthma.

TABLE IV

THE SEVERE ANEMIAS OF HUMAN CANCER: SP. GR. 1.030 TO 1.039. HEMOGLOBIN LESS THAN 39 PER CENT; RED BLOOD COUNT LESS THAN 2,000,000 CELLS; SEDIMENT VOLUME LESS THAN 19 PER CENT

CLINIC	AGE	SEX	DIAGNOSIS	GRADE	R.R.	R.S.	SP. GR. BLOOD
G-12	52	M	Ca. Stomach, perforated 6 days later				1.031
G- 2	57	M	Ca. Stomach				1.034
G- 6	39	M	Ca. Cardia of Stomach				1.036
G-14	55	F	Ca. Stomach				1.038

TABLE V

THE MODERATELY SEVERE ANEMIAS OF CANCER: SP. GR. 1.040 TO 1.044. HEMOGLOBIN 40 PER CENT TO 59 PER CENT; R.B.C. TWO TO THREE MILLION CELLS. SEDIMENT VOLUME 20 PER CENT TO 29 PER CENT

CLINIC	AGE	SEX	DIAGNOSIS	GRADE	R.R.	R.S.	SP. GR. BLOOD
D-69	75	F	Adenoca. Rectum	III	Yes		1.040
E-49	47	F	Primary Advanced Ca. Cervix				1.040
H-15	59	F	Neurogenic Myxosarc. of back	II	Yes		1.041
E-23	20	F	Rectovaginal Ca. (extensive)	III			1.041
C- 2	53	F	Lipo-sarc. rt. deltoid.				1.041
E-41	48	F	Sqam. ca. Cervix	II	Yes		1.042
E-37	43	F	Ca. Cervix				1.043
E-28	56	F	Ca. Cervix Recurrent postoperative				1.044
E-31	45	F	Ca. Cervix				1.044
D- 9	67	F	Adenoca. Rectum	I	Yes		1.044
D-56	32	F	Adenoma malignum. Rect.	II			1.044

TABLE VI

THE MILD ANEMIAS OF HUMAN CANCER: SP. GR. 1.045 TO 1.049. HEMOGLOBIN 60 PER CENT TO 79 PER CENT; R.B.C. THREE TO FOUR MILLION CELLS; SEDIMENT VOLUME 30 PER CENT TO 39 PER CENT

CLINIC	AGE	SEX	DIAGNOSIS	GRADE	R.R.	R.S.	SP. GR. BLOOD
G-11	56	M	Ca., Pancreas (Diabetic) Cholecystogastrostomy 3 mo. prior				1.045
I- 3	24	M	Teratoma Testis: Embryonal Ca.				1.045
I- 5	38	M	Pap. Ep. Ca. Bladder	I	Yes		1.045
D-36	43	M	Aden. Malig. Rectal	II	Yes		1.045
A-27	62	F	Ca. Breast, Postop.				1.046
E-38	52	F	Pap. Ep. Ca. Cervix	II		Yes	1.046
D-31	38	F	Gelatinous Aden. Ca.	III & IV	Yes		1.046
D-37	32	M	Malig. Polyp. or Pap. Ep. Ca.	III			1.046
F- 2	74	M	Ca. Mucosa Cheek				1.046
F- 5	15	M	Anaplastic Ca. Nasophar.	III		Yes	1.046
I- 7	70	M	Recurrent Ca. Bladder (prostatectomy, 2 yr. before)				1.046
I-12	68	M	Ad. Ca. Prostate				1.046
D-50	58	M	Adenoma Malig. Rectal	II	Yes		1.047
D-80	47	M	Adenoca. Rectum (infiltrated)	III	Yes		1.047
D-92	55	F	Gelat. Ad. Ca. Rectum	III	Yes		1.047
C-44	29	M	Recurrent Neuro. Sarc.				1.047
G- 1	40	M	Ca. Stomach				1.047
G-18	71	M	Ca. Stomach				1.047
H- 4	50	F	Myxosarcoma rt. leg				1.047
I- 4	61	M	Ep. Ca. Bladder	III		Yes	1.047
I-11	68	M	Ad. Ca. Prostate	II	Yes		1.047
D-23	64	M	Ad. Malignum, Rectum (infiltrated)	II	Yes		1.048
D-58	70	M	Ad. Malignum, Rectum	II	Yes		1.048
D-93	45	M	Ad. Malignum, Rectum (infiltrating)	II	Yes		1.048
A-32	63	F	Ca. Rt. Breast and Ca. Corp. Ut. Preop.				1.048
A-35	60	F	Ca. Breast, Preop. Radiated				1.048
C-24	67	M	Recurrent Neur. Sarcoma				1.048
G- 3	48	M	Ca. Stomach Adv. inoperable. Cardia				1.048
G- 4	62	M	Ca. Stomach, Fundus				1.048
H- 8	11	F	Osteogenic. Sarc. Femur.				1.048
I- 9	48	M	Ad. Ca. Prostate, w. Bone met.				1.048
I-16	45	M	Teratoma Testis				1.048
B-72	63	M	Superficial Sq. Ca.	I	Yes		1.049
C-18	62	M	Spindle cell sarcoma				1.049
C-40	72	F	Fibrosarc. lower extrem.				1.049
C-73	51	M	Neurosarcoma				1.049
D- 2	50	F	Pap. Ad. Ca. Rectum	III	Yes		1.049
D-19	73	M	Ad. Ca. Rectum	III	Yes		1.049
D-26	73	M	Ad. Malig. Rectum	II	Yes		1.049
D-32	44	M	Colloid Ca. Rectum	II	Yes		1.049
D-33	51	M	Ad. Malignum Rectum				1.049
D-91	66	F	Colloid Ca. Rectum (infiltrated)	II			1.049
D-95	60	F	Ad. Malignum. Rectum (extensive with chr. inflam.)	II			1.049
D-97	46	F	Ad. Ca. Rectum	III	Yes		1.049
E-39	29	F	Ca. Cervix				1.049
F-28	62	M	Sq. Ca. Lip	I	Yes		1.049
G- 7	62	F	Ad. Ca. (Pancreas?) Cyst				1.049
G-16	50	M	Ca. Stomach (with rt. subphrenic abscess)	III	Yes		1.049

of the radiosensitivity of the cancerous growths in the last 300 cases observed. Table VIII represents the cases of anemia found in the noncancerous group of lesions.

In the series of 23 cases I reported in 1929, ten, or 43 per cent, fell into the anemic group. This is in striking contrast to the present larger series of 400 observations where the corrected incidence of anemia was found to be 22 per cent. This may be explained not alone by the greater accuracy obtained in observing a larger series, but also by the fact that the smaller series was observed in hospitalized patients, usually late stages of cancer, whereas the larger series was compiled almost wholly (with the exception of about 6 cases) from ambulatory, out-patient cases. This low incidence of the anemias associated with early, ambulatory cases of cancer emphasizes the belief that cancer, at least in its beginnings, is a local disease. The 22 per cent incidence compares favorably with the 30 per cent incidence of anemias associated with neoplasms other than those of cancer; it contrasts strikingly with the 42 per cent of combined hospital and clinic patients (85 cases) of a general hospital as reported by Polowe,[6] and still more strikingly with the 52 per cent of purely hospital cases as noted in Table III.

This low incidence of anemia of 22 per cent bears further comment. It tends to minimize the current idea that irradiation of the cancerous growths produces anemia. I do not have the exact data as to how many of the observed cases were under radiation treatment, but I do recall that most of them had received some such treatment.* Lucas[7] feels that this factor as a cause of anemia is overemphasized. Examination of Tables IV, V, and VI tends to bear this out. Only 1.4 per cent of the observations (Table I) fell into the group of severe anemias. All four were in cancer of the stomach. Only 3.8 per cent of the observations fell into the moderately severe group of anemias. The majority of these were associated with cancer of the cervix and of the rectum. The balance of 16.8 per cent fell into the mild group of anemias. The lesions encountered were mainly those of the gastrointestinal tract, and those of the genitourinary tract (urinary bladder, testis, cervix). All of these three groups of anemias can be plausibly explained on a clinical basis (the presence of large ulcerated areas from which there is a constant oozing of blood and through which bacteria gain easy access to the body). Starvation and dehydration are no mean factors in the cause of anemia associated with cancer of the gastrointestinal tract. Irradiation should not, therefore, be considered an important factor in the production of anemia.

Starvation, hemorrhage, and infection form the great etiologic triad in the production of the anemias associated with cancer. The nature of this study (i.e., the single observations and the lack of follow-up) prevents an accurate interpretation of the blood status case by case. However, the number observed, plus some clinical note on each case, plus clinicopathologic follow-ups on some of the cases enable me to give a fair cross-section of my clinical impressions as

*My notes show that in the breast series 18 cases (18 per cent) were treated by some form of irradiation. Of these only one fell into the anemic group (mild, with specific gravity of 1.048). Sixteen had blood densities within normal limits. One had a blood density of higher than normal, 1.061, and had received treatment with radium tubes and high voltage cycle.

to the factors determining the variation of the percentage of anemias in the various clinics. Thus two main groups stand out: (a) Cancer of the easily visible portions of the body as found in the breast, skin, oral, and gynecologic clinics. It is plausible to assume that the percentage of anemias is less in this group since the lesions are discovered earlier by the patient and are, therefore, treated earlier. (b) Cancer of the hidden portions of the body as found in the gastrointestinal and urologic clinic. It is plausible to assume that the percentage of anemias is greater in this group as the patient usually discovers his condition when the growth has advanced and usually long after starvation, hemorrhage, and infection have made inroads into his body economy.*

In the sarcomas attention is invited only to the fact that those involving bone (a blood-forming organ) exhibit at least half again a greater incidence of anemia than do those involving the nerves.

The group of higher than normal blood densities shown in the last two columns of Table I deserves comment. I cannot explain the high blood density in all the cases, but my clinical notes indicate that venestasis may be an important etiologic factor. Thus of the 13 breast cases one patient was noted as being inoperable, one was noted as having edema, and 7 were postoperative cases with possible interference of venous return, though my notes do not specifically show that in these 7 cases. In the group of 5 patients with epitheliomas, one patient with a specific gravity of 1.062 is noted as having a squamous carcinoma with metastasis to the axillary nodes, one patient with a specific gravity of 1.064 is noted as having a squamous carcinoma with clubbed finger tips, the other three patients are noted as having squamous carcinomas on hands and arms. In the neurogenic sarcoma group, of the 4, one involved the left forearm and the other a right lower extremity, but my notes do not indicate that there was involvement of the regional lymph notes, which if present might account for an increased blood density. One melanosarcoma is noted as involving the right axilla. One extensive angiosarcoma of the back was associated with a specific gravity of the blood of 1.062.

In this group of higher than normal blood densities, it is the negative phase which compels attention. Thus, no high blood densities appear in any of the gastric cases. It is in this clinic that I saw cases of dehydration where one would expect a high blood density (especially in view of the normal or nearly normal red blood cell counts and hemoglobin values that were found). But such was not the case. Other factors, such as loss, by emesis, of ash content of the blood, and factors as yet undetermined tend to produce a blood density more in keeping with the clinical picture. The same is true of cancer of the large bowel where only one case (1 per cent) was found to have a blood density of 1.061.

It must be borne in mind that the complexities of the forces at play, such as the fluctuating water and mineral balances and the destroyed rhythm of blood formation, tend at times to produce normal specific gravity values while pathologic forces are destroying the patient. The importance of clinical judgment is obvious.

*A striking example of this was seen in Case G-12, Table IV, carcinoma of the stomach in a male, aged fifty-two, wherein a blood density of 1.031 (the lowest density observed in the entire series) was found six days prior to perforation of the stomach.

Just a note in passing on the benign group of higher than normal blood densities. One case only deserves comment, that of a boy of nineteen, with a bone cyst, wherein the specific gravity of the blood was 1.061. This high blood density may have been due to an increased calcium content of the blood. The high specific gravity of the blood may be of differential diagnostic value in such cases.

(c) *Grading and Sensitivity.*—In Table VII one sees that 20 per cent of the cases graded fell into the anemic group, the more malignant grades (II and III) claiming the greatest number. This 20 per cent is in keeping with the 22 per cent incidence of the anemias in the 400 cases observed. Of the radioresistant lesions 21 per cent fell into the anemic group. Here again the incidence is in keeping with the 22 per cent for the entire series. Of the radiosensitive lesions 10 per cent fell into the anemic group. No radiosensitive lesions were found in the severe and moderately severe groups of anemias (Tables IV and V). It appears quite likely that the low incidence of radiosensitive lesions among the anemias is due to their rapid growth and consequent early discovery by the patient who presents himself for treatment before anemia has set in.

TABLE VII

GRADING AND SENSITIVITY REPORTS REVIEWED IN 300 CASES OF HUMAN CANCER

Total graded as to malignancy	148
Total qualified as to radiosensitivity	137
Total qualified and graded	131
Total graded but not qualified	17
Total qualified but not graded	11
Total neither graded nor qualified	152

	GRADE NONANEMIC: SP. GR. 1.050 OR OVER		ANEMIC: SP. GR. UNDER 1.050	
	NO. OBS.	PER CENT	NO. OBS.	PER CENT
I	21	18	4	13
II	65	55	14	47
III	30	26	12	40
IV	1	1	—	—
	117	80% of total graded	30	20% of graded
R.R.	84	79%	22	21%
R.S.	28	90%	3	10%

TABLE VIII

THE TEN ANEMIAS AMONG THE 50 CASES OF HUMAN NONCANCEROUS LESIONS

CLINIC	AGE	SEX	DIAGNOSIS	SP. GR. BLOOD
G- 6b	53	M	Achylia gastrica	1.043
I- 3b	68	M	Hyp. prostate postop.	1.043
I- 2b	66	M	Hyp. prostate postop.	1.044
I- 1b	77	F	Epithelial papilloma	1.046
E-13b	47	F	Fibroid uterus	1.047
E-10b	28	F	Fibroid uterus	1.048
G- 4b	53	M	Gastric ulcer	1.048
G- 5b	55	M	Gastric ulcer	1.049
G-11b	35	M	Mycosis fungoides (skin)	1.049
G-14b	78	M	Achylia gastrica	1.049

From a statistical point of view, therefore, radioresistance is only coincidentally associated with an anemic status.

In 23 of the 31 lesions estimated as being radiosensitive, qualifying terms such as "relatively radiosensitive" or "moderately radiosensitive" were used. The estimate of sensitivity made by the pathologist appears to be ultimately conditioned by the size, location, and accessibility of the growth. Borak[13,14] terms these the conditional and constitutional factors, the conditional factors being determined by the character of the surrounding tissues, including regional and distant organs; the constitutional factors being determined by the nature of the tumor and of its matrix. It should be noted that Borak qualifies his conclusions by stating that, in addition to these biologic factors, the rôle of other clinical factors and the physical properties of the rays must be considered for prognosis and technic of irradiation. Pack and Quimby[15] state that radioresistance is a latent property of cancer tissue. They cite Canti as properly suggesting that "in the case of neoplasms there may be an indirect factor playing an important part." The nature of this indirect factor is not mentioned. Nemenow[16] feels that since the roentgen rays influence the metastructure of the cells, the molecules and atoms, it is as a rule impossible to estimate the radiosensitivity on the basis of the histologic picture that reveals only the gross structure of the cell. To me, this seems like a hint that cellular chemistry may be a factor of influence.

Several men have thought it likely that the blood status influenced the sensitivity of the tumor process. Dean,[17] ten years ago, made the statement, "The relationship between our skin tests, the therapeutic results and the patients' blood quality has been so close as to warrant the prediction of the effect of therapy within certain limits on the basis of the latter factor alone." Dean found that the usual skin erythema dose had no effect on the anemic patient. After blood transfusion in such a case, Dean obtained a skin erythema with the usual dose. More recently, Stewart[18] very clearly presented the status of estimated sensitivity and observed results of irradiation of pathologic tissues. He stated, "The entire situation suggests the presence of some entirely unknown factor or factors influencing the sensitivity of the tumor process." Stewart further stated that, "After all, the only law of radiosensitivity which remains essentially unchallenged is that one accomplishes very little by irradiating a cachectic patient, unless the cachexia results from a highly sensitive tumor of the type of lymphosarcoma, thymoma, Wilms's tumor and the like." My own impression (stated here merely as a matter of record and as a subject for future investigation) is that there is some underlying electrolytic factor in the blood, whether the patient is anemic or not anemic (I have in mind a chloride retention), which in some way hinders the selective action of the rays. This impression is based on the work of Stammler and Moraczewski,[19] Macht and Teagraden,[20] and on some of my own unpublished work. This remains to be proved. I feel that the anemia is only coincidental, and that when it is associated with a radioresistant lesion, it (the anemia) is then only a clinical expression of a modified electrolytic content of the blood and should be so treated (as by transfusion) before irradiation of the patient.

SUMMARY

1. The specific gravity of the whole blood was determined in 400 cases of proved cancer. A relatively low incidence of anemia was found: 22 per cent of the series as a whole; 5.2 per cent incidence of combined severe and moderately severe grades of anemia.

2. These anemias can usually be explained on a clinical basis as secondary effects of the cancer process and may so be treated.

3. Anemia appears to be only coincidentally associated with radioresistant lesions.

4. It appears that irradiation as a cause for anemia is overestimated.

5. Venestasis may be an important etiologic factor in the production of higher than normal blood densities.

6. The falling drop technic for determining the specific gravity of fluids offers an easy, rapid, and accurate orientation as to the status of the blood at any moment. It permits of limited differential diagnostic value in gastric lesions wherein low blood densities (expressive of severe or moderately severe grades of anemia) may make one lean toward a diagnosis of cancer.[6] In bone lesions, where hyperparathyroidism is suspected, higher than normal blood densities may add corroborative evidence.

The author wishes to express his appreciation to the various members of the staff of the Memorial Hospital, New York, for their kind assistance in obtaining the correct material and for the time given me in informal discussion of the work. The author alone is responsible for the opinions expressed in this paper. I wish also to thank Sarah Dimond Abrash, A.B., Paterson, N. J., for her aid in the recording of the data.

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LABORATORY METHODS

DETERMINATION OF THE STROKE VOLUME OF THE HEART*

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THERE is an occasional demand in routine laboratories to determine the stroke volume of the heart in connection with cardiac constriction cases. This may be obtained by determining the oxygen content of venous and arterial blood and the oxygen consumption of the lungs.

Principle.—The one-minute oxygen consumption of an individual at rest and fasting for several hours is determined using a basal metabolism apparatus. The pulse is recorded; the oxygen content of venous and arterial blood is determined. The stroke volume can then be calculated.

Method.—A. The oxygen consumption of the patient is determined by means of a basal metabolism apparatus. The temperature of the oxygen in the apparatus is noted, also the barometric pressure. The number of cubic centimeters of oxygen consumed per minute is converted to standard conditions by calculation.

B. About 5 c.c. of blood are withdrawn from a vein in the arm of the patient. The blood is protected from contact with air by using a Kaufman Luer double tip syringe, the side arm is connected by rubber tubing containing mineral oil to a test tube lined with approximately 25 mg. of sodium oxalate. A like amount of blood is withdrawn in a similar manner from an artery located in the wrist of the patient.

The pulse of the patient is recorded.

Sodium hydroxide solution: A 2 per cent solution of sodium hydroxide is aerated by introducing it into a separatory funnel and shaking it several minutes.

Oxygen reagent: Potassium ferricyanide 3 gm., saponin 3 gm., caprylic alcohol 3 c.c., and distilled water, a sufficient quantity to make 1,000 c.c.

Using a van Slyke and Cullen CO_2 Pipette, 7.5 c.c. of the oxygen reagent are introduced into the cup of the apparatus and deaerated by subjecting it to repeated negative pressures. When the reagent is gas-free, 6 c.c. are forced up into the cup of the apparatus. Two cubic centimeters of venous blood are transferred from under the oil in the test tube to the cup of the CO_2 pipette, slowly being delivered under the reagent simultaneously being permitted to enter the chamber of the pipette followed by 5 c.c. of reagent. The excess of reagent is removed from the cup of the pipette. A few drops of mercury are introduced to act as a stopcock seal.

*From the Laboratories of the Graduate Hospital, University of Pennsylvania.
Received for publication, August 5, 1933.

Most of the mercury is evacuated from the chamber of the pipette and the apparatus is shaken until the gases liberated from the blood by the reagent give a constant volume. (Shake from five to ten minutes.) The gases are oxygen, nitrogen, and carbon dioxide. A negative pressure is now produced in the chamber of the pipette by bringing the upper surface of the liquids to the 2 c.c. graduation. The cup of the pipette is rinsed with distilled water, and 0.5 c.c. of aerated sodium hydroxide is permitted to enter the chamber of the pipette followed by a few drops of mercury. One minute is allowed for the sodium hydroxide to combine with the carbon dioxide gas. The gases (oxygen and nitrogen) are now brought to atmospheric pressure and a reading taken. The temperature and barometric pressure are noted during the analysis.

C. The oxygen content of arterial blood is determined in the same way as that of venous blood.

Calculation.—

A. A—c.c. of O_2 consumed per minute (from basal metabolism apparatus)
P_1—mm. barometric pressure
P_2—760 mm.
T_1—Absolute temperature of gas
T_2—273 degrees Absolute

A.V.T. = Aqueous vapor tension

$$O_2 \text{ c.c. consumed per minute} = \frac{A \times T_2 \times (P_1 - \text{A.V.T.})}{T_1 \times P_2}$$

B. O_2 *in venous blood.*
B = c.c. of $O_2 + N_2$ in blood.

$$\text{Volume per cent } O_2 = \left[\frac{P_1 - \text{A.V.T.}}{760\ (1 + 0.00367 \times {}^\circ\text{C})} \times 50 \times B\right] - 1.36$$

1.36 = Volume per cent of N_2

C. O_2 *in arterial blood.*
Use the same formula as for oxygen in venous blood.

D. $$\text{Minute volume of blood in cubic centimeters} = \frac{100 \times \text{c.c. } O_2 \text{ consumed per minute}}{\%\ O_2 \text{ in ``C'' } - \%\ O_2 \text{ in ``B''}}$$

$$\text{Stroke volume in cubic centimeters} = \frac{\text{Minute volume of blood in c.c.}}{\text{Pulse}}$$

Normal stroke volume for patient at rest is from 50 to 100 c.c. in each ventricle per beat.

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THE PREPARATION AND USE OF COLLOIDAL CARBON SOLUTIONS*

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DURING an investigation undertaken a number of years ago, by the senior author, of the chemical changes in the spinal fluid responsible for the flocculation of the gold sol in the colloidal gold test, it was found that no correlation could be drawn between the two factors because of the variability of the gold sol.

It was found that two gold sols, even though prepared at the same time with the same reagents under the same conditions, did not give identical curves with the same spinal fluids. It was further found that one could not keep a satisfactory gold sol for a sufficiently long time to carry out a series of experiments which would extend over a period of months. In order to carry out the investigation as planned, it was necessary either to have a colloidal solution which could be made in large amounts that would keep indefinitely, or else to have one which could be made at different times and still yield identical results with spinal fluids.

As none of the colloidal solutions then being used could satisfactorily meet these requirements, a search was made for other colloids. After a number of solutions were investigated, it seemed that a colloidal suspension of carbon offered most promise of fulfilling the requirements. A preliminary report on the preparation and use of colloidal carbon solutions was presented at the meeting of the Federation of American Societies for Experimental Biology in 1927.[1] In 1928, at the Philadelphia meeting of the Interurban Clinical Club, a paper was presented giving results of further studies with the method. At this time it was pointed out that solutions of India ink would give similar results, although the reagent was not as sensitive as the electrolytic ones.

A further demonstration of this method was made at the International Physiological Congress at Boston in 1929. At that time a carbon solution made three years previously was used with spinal fluids obtained through the courtesy of Dr. Frank Fremont Smith of the Department of Neurology at Harvard Medical School. The carbon curves in every instance agreed with those obtained independently in his laboratory by the colloidal gold test. The curve of patient M. B. in Table VI was obtained with this solution. Recently Schube and Harms[2] have published an article confirming in part these findings, although no reference was given as to the original investigations of Looney.

*From the Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital.

Received for publication, August 1, 1933.

METHOD

The preparation of colloidal solutions of carbon is readily accomplished by means of electrolysis between two carbon electrodes in a faintly acid medium. Solutions prepared by acid alone are as a rule not stable, but the addition of an oxidizing agent such as potassium dichromate increases their stability.

The carbon electrodes are obtained from discarded dry cells and it is preferable to use electrodes which have not been impregnated with paraffin. These are connected in series with a 22 ohm rheostat, capable of handling 5 amperes, an ammeter and the terminals of a 110 volt source of direct current. A volt meter is connected across the two electrodes which are immersed in a jar containing three liters of water to which has been added 3 ml. of concentrated sulphuric acid saturated with potassium dichromate. The desired amperage and voltage were obtained by regulating the distance between the electrodes and by manipulating the rheostat. Current was passed through the solution for from ten to twenty hours, yielding a black solution of carbon in colloidal suspension. After standing for several days the clear supernatant solution was decanted from the larger particles which had settled out. A good solution should be clear, dark brownish in color, and of such concentration that print can be distinguished through a layer 5 or 6 mm. in thickness. This depth of color can be obtained by diluting with distilled water. Pale solutions should be discarded.

When added to spinal fluids, varying degrees of precipitation may be observed and utilized as with colloidal gold solutions to yield a curve. The following scale has been used in recording observations:

0—no change
1—no precipitate, change in color
2—light precipitate, deep color
3—heavy precipitate, marked color
4—heavy precipitate, faint color
5—completely colorless

Eleven tubes were set up as for colloidal gold curves, 1 ml. of 1.0 per cent sodium chloride solution placed in each tube, and 1 ml. of spinal fluid added to the first tube. After thorough mixing 1 ml. was transferred from the first tube to the second, mixed thoroughly, and the process repeated to give a series of dilutions ½, ¼, ⅛, etc., the 1 ml. for transfer from the tenth tube being discarded, and the eleventh tube acting as control. Two ml. of the colloidal carbon solution were added to each tube, the racks shaken and allowed to stand overnight.

In Table I are given some of the preliminary experiments carried out during 1927 and 1928. It will be noted in Case 1 on March 15 that different carbon solutions give somewhat different results although the curves are of the same type. The character of the curve changed as the patient recovered so that it approached that of the normal in which precipitation occurs only in the first two tubes. That the curve obtained in meningitis differs in character from that obtained in general paresis is also evident from this table.

Although these cases showed that colloidal carbon solutions appeared to be suitable as a test medium, it was felt that a thorough study of all the factors influencing the sensitivity of the solutions was needed before the method could be used clinically.

The sensitivity of the reagent is influenced by the conditions of preparation and also by the salt concentration and P_H of the solution used to make the spinal fluid dilutions. Solutions were prepared using a current density of 2, 3, and 4 amperes at potentials of 20, 30, and 40 volts.

It was found that solutions prepared at the higher voltages were as a rule too unstable for use. Thus, of 7 samples made at 40 volts, 6 precipitated out completely after a few days' standing, and the seventh was so sensitive that the addition of 1 per cent salt solution caused complete precipitation. The samples prepared at very low voltages and amperages did not as a rule contain sufficient carbon to make them available for study. Stable solutions

TABLE I

NO.	PATIENT	DATE	SAMPLE	CARBON CURVE	DIAGNOSIS
1	W. L.	3/ 8	D	1155531000	Meningitis
		3/15	D	1115551000	Meningitis
		3/15	E	1145552000	Meningitis
		3/15	G	1135554320	Meningitis
		3/20	G	55430000000	Meningitis
		3/21	G	5430000000	Meningitis
2	----	3/19	G	5554000000	Meningitis
3	J. H.	4/15	G	2445544321	
		4/18	G	1155554332	Meningitis
4	T. W.	4/15	G	000555432	Meningitis
		4/18	G	000555543	Meningitis
5	C. S.	9/16	D	1255544330	Meningitis
		9/16	E	2345511000	Meningitis
6	M. B.			00235544320	Tuberculous meningitis
7	----	3/25	G	5400000000	Maternity, normal
8	----	3/20	G	5400000000	Normal
9	S. H.	6/13	D	2100000000	Normal
10	F. P.	4/ 4	G	55543210000	Wassermann positive G.P.
11	----	6/13	D	5543211000	Wassermann positive G.P.
12	J. N.	7/19	D	5543200000	Wassermann positive G.P.
13	J. H.	9/18	D	5443222110	Wassermann positive G.P.

could be prepared at from 20 to 30 volts and from 3 to 4 amperes. The solutions made at 4 amperes were considerably more sensitive than those made at 3 amperes. The samples prepared with electrodes having a small surface area immersed in the solution were on the whole more sensitive than those having the same voltage and amperage but with a greater area of electrode in the bath. The best preparations are obtained with fresh electrodes immersed to a depth of from 13 to 14 cm. at 30 volts and 3 amperes. These solutions contain the maximum amount of carbon, about 250 to 270 mg. being contained in each 100 ml.

Variation in the sensitivity and stability of the solutions can be obtained by altering the salt concentration of the solution used in making the spinal fluid dilutions. This influence of the salt solution upon the sensitivity of the reaction is shown in Table II, which records the data when the dilutions were

made with distilled water, 1.0 per cent, and 1.5 per cent sodium chloride solutions. Increase in the salt concentration increases the sensitivity.

The stability of the carbon solutions on standing is a function of the sensitivity of the colloid. The less sensitive solutions can be kept for long periods, as is shown by the fact that the solution used for demonstration at the International Physiological Congress in 1929 had been prepared three years previously. On the other hand, extremely sensitive solutions have a tendency to precipitate out so that they are too light to use. Thus we find that Sample 6, when three weeks old, did not precipitate on the addition of two volumes of carbon to one volume of 2 per cent salt solution, but at six

TABLE II

NO.	PATIENT	DATE	DILUTION	CARBON CURVE
13	J. H.	9/18	Water	5410000000
			1.0% NaCl	5443222110
14	A. D.	9/18	Water	5400000000
			1.0% NaCl	5442000000
15	P. K.		1.5% NaCl	4554321110
			1.0% NaCl	5542100000
16	H. C.		1.5% NaCl	5443211000
			1.0% NaCl	4431000000

months it was precipitated by 1.4 per cent salt but not by 1.2 per cent salt solution. This increase in sensitivity on standing is not sufficient in the case of the better solutions to prevent their being used for many months.

After the carbon has been added to the spinal fluid dilutions the readings can be made after standing overnight. Standing for a longer period has no effect on the curve obtained, as is shown by the readings taken at twenty-four hours and forty-eight hours in Table III.

TABLE III

NO.	PATIENT	DATE	HOURS	CARBON CURVE
15	P. K.	11/25	24	5542100000
			48	5543100000
16	H. C.	11/25	24	4431000000
			48	4431000000

The solution as made is approximately 0.033 normal and is quite acid, various samples yielding P_H values by the quinydrone electrode from 1.5 to 1.8. The influence of changes in acidity upon the sensitivity of the reaction was examined, the data of Table IV showing the influence of sodium carbonate and sodium hydroxide upon the precipitation of carbon by sodium chloride. The dilutions in Table IV were made by varying the concentration of sodium carbonate and sodium hydroxide in 2 per cent sodium chloride solution, using the 2:1 carbon: saline ratio as noted for the effect of salt concentration.

It will be seen that gradually increasing the amount of alkali added decreases the precipitation of the carbon solutions at first but finally the mixture again precipitates so that the addition of from 2 to 3 c.c. of 10 per cent

TABLE IV

SAMPLE C.C. ADDED	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0
A. Stabilizing Effect of Addition of 10 Per Cent NaOH to 2 Per Cent NaCl Solution											
6	0	0	0	0	0	0	0	0	2	2	3
23	2	0	0	0	0	0	0	2	4	5	5
24	2	0	0	0	0	0	1	4	5	5	5
26	0	0	0	0	0	1	3	5	5	5	5
27	2	2	2	0	0	0	2	3	4	5	5

SAMPLE C.C. ADDED	1.50	1.35	1.20	1.05	0.90	0.75	0.60	0.45	0.30	0.00
B. Stabilizing Effect of Addition of 10 Per Cent Na_2CO_3 to 2 Per Cent NaCl Solution										
6	0	0	0	0	0	0	0	0	0	4
23	0	0	0	0	0	0	0	1	4	5
24	0	0	0	0	0	2	2	4	5	5
26	0	0	0	0	0	0	0	0	0	4
27	0	0	0	0	0	1	2	4	5	5
28	0	0	0	0	0	0	0	0	1	1

sodium hydroxide causes complete precipitation. The zone in which there is no precipitation is much wider for sodium carbonate than for sodium hydroxide. This stabilizing effect of the addition of alkali can be utilized to adjust oversensitive carbon solutions, so that they can be made suitable for use with spinal fluids. This effect is given in Table V, in which the curves obtained by the addition of varying amounts of sodium carbonate to the colloidal carbon solutions are compared with the corresponding gold curve. The milliliters of 10 per cent sodium carbonate solution added to 100 ml. of colloidal carbon are recorded as the second value in the hyphenated sample number, e.g. 26.-0.2 indicates that 0.2 ml. of 10 per cent sodium carbonate was

TABLE V

EFFECT OF ADDING 10 PER CENT Na_2CO_3 ON THE CURVES OBTAINED FROM SPINAL FLUIDS

SAMPLE AND C.C. ADDED	CARBON CURVE	GOLD CURVE
26–0.0	5544320000	4443210000
26–0.0	4433220000	1110000000
26–0.2	5421110000	5555310000
26–0.2	5420000000	1111000000
26–0.5	0000000000	0000000000
26–0.5	0000000000	4332100000
6–0.0	5542100000	5555353210
6–0.0	4300000000	1111000000
28–0.0	5542100000	5555432100
28–0.0	5300000000	1111000000
28–0.5	5400000000	4433210000
28–0.5	4000000000	2222100000
23–1.0	5554433200	5555532100
23–1.5	5554333322	5555532100
23–1.5	5554432210	1111000000
23–1.8	5432100000	5555310000
23–1.8	4210000000	0000000000
23–2.0	0000000000	5554210000
27–1.0	5544333222	5544321000
27–1.8	5532100000	5555532100
27–1-8	2411000000	1111000000
27–2.0	4322220000	5544321000

TABLE VI

PATIENT	DATE	WASS.	CELL	GLOB.	SUGAR	PROT.		GOLD CURVE	CARBON CURVE
M.B.*	1929	----	440		24	269	0.511	0001123321	00235544320
				Carbon Sample 6					
H.C.	11/21	pos.	8(RBD)	ST	112	65	0.714	5555555544	5532100000
P.K.	11/21	pos.	155	VST	77	105	0.708	555554321	5543200000
L.G.	11/14	pos.	7(RBD)	T	62	74	0.588	5555353210	5542100000
G.F.	11/ 7	pos.	4(RBD)	VST	75	59	0.628	4555533210	5443100000
R.S.	1/ 2	pos.	7(RBD)	VST	79	51	0.719	4555521000	5544322100
L.M.	11/30	pos.	4	ST	46	53	0.717	4554321000	5543210000
E.G.	11/30	pos.	8	T	47	55	0.723	5553210000	5443210000
A.A.	11/30	pos.	6	ST	44	50	0.723	5532100000	5433200000
M.G.	1/ 2	pos.	7(RBD)	VST	74	83	0.734	4444422100	5544333222
A.C.	11/30	doubt.	1	VST	87	32	0.728	2221000000	4332100000
F.B.	11/14	neg.	4	SPT	66	33	0.602	1111110000	4300000000
F.S.	11/14	neg.	1	SPT	102	45	0.602	1111100000	5420000000
R.D.	11/14	neg.	2	VST	70	37	0.624	1111000000	4300000000
A.C.	11/14	neg.	1(RBD)	SPT	77	34	0.620	0001110000	3100000000
R.B.	11/21	neg.	6	0	109	44	0.713	0000000000	4200000000
A.B.	11/21	neg.	4	VST	110	35	0.712	0000000000	3000000000
J.J.	11/21	neg.	5(RBD)	ST	110	30	0.722	0000000000	5300000000
F.B.	11/15	neg.	3	0	54	43	0.625	0000000000	3200000000
				Carbon Sample 28					
F.G.	2/13	pos.	2	T	59	98	0.727	5555532100	5531110000
H.S.	2/20	pos.	9	ST	72	78	0.714	5555432100	5542100000
R.G.	2/27		3(RBD)	VST	72	47	0.743	5443210000	5421000000
F.J.	2/13	pos.	1	VST	65	56	0.727	4443210000	5322100000
J.U.	2/13	pos.	2	VST	68	53	0.722	3332100000	5320000000
J.U.	2/20	doubt.	1	T	86	80	0.740	2222100000	5410000000
C.C.	2/20	neg.	9	VST	81	38	0.678	2221100000	4100000000
J.V.	2/13	neg.	1	ST	79	65	0.723	1111000000	5310000000
R.S.	2/20	neg.	1	VST	79	40	0.709	1111000000	3200000000
J.G.	2/20	neg.	0	SPT	68	48	0.722	1111000000	4200000000
J.J.	2/27		5(RBD)	ST	78	38	0.734	1111000000	5300000000
A.H.	2/13	neg.	1	SPT	53	30	0.702	1110000000	3100000000
				Carbon Sample 29					
F.G.	2/13	pos.	2	T	59	98	0.727	5555532100	5543220000
H.S.	2/20	pos.	9	ST	72	78	0.714	5555432100	5543321000
J.U.	2/20	doubt.	1	T	86	80	0.740	2222100000	5432100000
C.C.	2/20	neg.	9	VST	81	38	0.678	2221000000	3221000000
R.S.	2/20	neg.	1	VST	79	40	0.709	1111000000	3320000000
J.G.	2/20	neg.	0	SPT	68	48	0.722	1111000000	4320000000
				Carbon Sample 27-2					
A.P.	2/ 7	pos.	8	VST	65	50	0.707	5544321000	4322220000
F.J.	2/13	pos.	1	VST	65	56	0.727	4443210000	4542100000
J.U.	2/13	pos.	2	VST	68	53	0.722	3332100000	5543322000
A.P.	2/ 7	neg.	3(RBD)	VST	61	51	0.700	2222110000	3221100000
J.L.	2/13	neg.	2	SPT	58	44	0.730	2222100000	3441000000
				Carbon Sample 27-1.8					
F.G.	2/13	pos.	2	VST	59	98	0.727	5555532100	5532100000
J.L.	2/13	neg.	2	SPT	58	44	0.730	2222100000	0000000000
J.V.	2/13	neg.	1	ST	79	65	0.723	1111000000	2411000000
				Carbon Sample 15					
L.G.	11/14	pos.	7(RBD)	T	62	74	0.588	5555353210	5554200000
F.B.	11/14	neg.	4	SPT	66	33	0.602	1111110000	5531000000
R.D.	11/14	neg.	2	VST	70	37	0.624	1111000000	5420000000
A.C.	11/14	neg.	1(RBD)	SPT	77	34	0.620	0001110000	5531000000
				Carbon Sample 23-1.8					
E.A.	2/27		5	VST	65	34	0.724	5555310000	5432100000
C.M.	2/27		1	SPT	22	30	0.659	0000000000	4210000000

*Tuberculous Meningitis.

added to each 100 ml. of carbon Sample 26. It is evident that the addition of carbonate changes the character of the colloidal suspensions, so that it is possible to regulate the sensitivity in such a way that the carbon solutions will give curves very similar to those obtained with the gold sols.

In Table VI we have recorded the complete data for 54 spinal fluids. It will be noted that the carbon curves parallel those of the gold sol. In the normal spinal fluids there is as a rule precipitation only in the first two tubes with the carbon solutions, whereas slight changes occur in many of the gold dilutions. In the fluids obtained from patients suffering from meningitis, the carbon curves offer still more striking contrast to those obtained with normal fluids inasmuch as the former show a protective action in the first tubes of the series.

The observations recorded in the foregoing tables are such as to indicate that colloidal carbon solutions can be prepared which may be used as test media for spinal fluids. The development of a standard technic to cover all possibilities demands further experimentation with tests of many spinal fluids and many different carbon samples, and will be the subject of a future paper.

SUMMARY

Colloidal carbon solutions suitable for use with spinal fluids can be prepared by electrolysis between two carbon electrodes in a 0.033 N Chromic acid bath.

The optimum sensitivity and stability is obtained at 30 volts and 3 amperes.

The sensitivity of the colloid can be altered by the addition of small amounts of 10 per cent sodium carbonate solution.

The curves obtained with colloidal carbon solutions are shown to be similar to those obtained with the gold sols.

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A METHOD FOR THE SEPARATION OF THE PRINCIPAL CONSTITUENTS OF BILE*

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INTRODUCTION

QUANTITATIVE studies of the various constituents of bile have been a matter of considerable difficulty. Bile is one of the most complex and variable of body fluids. Whipple[1] has pointed out the wide variety of chemical compounds which may be found. Wide ranges in the concentration of any constituent may occur, in biles from the same species, in liver and in gallbladder bile from the same animal, and in addition, there is a marked difference in composition from one species to another.

Three of the most important groups of compounds which occur in all types are the pigments, the bile salts, and the sterols. The bile pigments include bilirubin, biliverdin, and other pigment derivatives. The bile salts form one of the most complex groups of compounds which occur biologically. Most animal biles contain some or all of the conjugated bile salts, combinations of glycine or taurine with cholic or deoxycholic acids. In addition, other bile acids may occur which are more or less characteristic of the species. Human bile, for example, may also contain lithocholic and chenodeoxycholic acids (Ingold[2]). By virtue of their complex nature certain bile acids have a marked tendency to form more or less stable complexes; deoxycholic acid with fatty acids forming the choleic acids; complexes have also been postulated with lecithin and with cholesterol. The bile sterols include free and ester cholesterol and probably also oxycholesterol.

The physical and chemical properties of these constituents are such that even a trace of one compound may interfere with the accurate quantitative estimation of another. Two examples of such interference will illustrate the difficulties.

The estimation of cholesterol has presented special difficulties. Not only is it necessary to prevent the removal of any bile salts during the extraction of cholesterol from a sample of bile, but also no pigments must be in the final extract. The presence of a tinge of yellow, due to the extraction of a trace of some pigment derivative as reported by several workers (McLure and Mortimer,[3] McMaster[4]), will interfere markedly with the accuracy of the final colorimetric estimation.

The Pettenkofer reaction has commonly been used for the estimation of

*From the Departments of Biochemistry and Experimental Surgery, McGill University, Montreal.

Received for publication, September 12, 1933.

This work has been conducted under a grant from the Department of Experimental Surgery.

the bile acids although only acids of the cholic group may be thus estimated (Reinhold and Wilson[5]). Bile pigments again interfere with the production of the characteristic color and hence with the accuracy. Attempts have been made to overcome this defect by selective adsorption of the interfering substances (McLure, Vance and Greene,[6] Aldrich and Bledsoe[7]). The deficiencies of such methods have been pointed out by Gregory and Pascoe[8] who have advocated a monochromatic light to eliminate the interference in the final colorimetric estimation.

It is obvious that a greater accuracy could be expected in such colorimetric estimations if the desired constituents were separated completely from all other substances in the specimens of bile. Selective adsorption provides such a means of separation. Bile pigments, for example, have been adsorbed from urine on calcium carbonate, Hooper and Whipple,[9] and on barium sulphate (Cole,[10] Hunter[11]).

Few applications of such inorganic adsorptions to bile have been reported. McLure and others[12] adsorbed the bile pigments from duodenal contents on calcium sulphate. Wit[13] has recently studied the effectiveness of such compounds and found that ferric hydroxide and aluminum hydroxide completely clarified bile. Chiray and Cuny[14] have recommended neutral lead acetate for this purpose. Peoples,[15] more recently, has used calcium hydroxide to remove substances other than bile salts from bile prior to their estimation.

An extension of the principle of selective adsorption and preferential extraction should make it possible to separate several constituents from the same specimen of bile so that each might be determined. The method detailed below provides a simple method for the separation of the bile pigments, bile acids, and cholesterol from the same specimen of bile.

Briefly, the bile pigments are adsorbed on zinc hydroxide in aqueous solution. After many adsorbents had been tried this proved the most satisfactory since the reagent completely adsorbed the pigments at a slightly alkaline reaction. This is essential since the free bile acids precipitate out in an acid medium. Zinc hydroxide has the added advantage that it is a protein precipitant.

The greater part of the bile salts remain in the supernatant liquid. The fraction which is loosely adsorbed on the zinc hydroxide precipitate is removed by extractions, first with water, then with ethyl alcohol, while the pigments are not so removed. The latter solvent also extracts the cholesterol. In order to prove the completeness of extraction it was assumed that all bile acids were extracted similarly although, as Cuny[16] pointed out, the oxyacids give little or no color with the reagents for the Pettenkofer reaction. A negative test, especially using the more sensitive modification of an alcoholic solution of the bile salts with furfuraldehyde and sulphuric acid, was accepted as indicative of complete removal of the bile salts.

Repeated tests have confirmed the fact that the more common bile salts, at least, are insoluble in petroleum ether. The other common solvents such as chloroform, carbon tetrachloride and ethyl ether tended to extract traces of substances which produced a yellowish or brownish color with the reagents for

the Liebermann-Burchard reaction for cholesterol. This solvent was recommended by Elman and Taussig[17] for extracting cholesterol from whole bile. A negative Liebermann-Burchard test was accepted as indicating complete extraction of the cholesterol. The quantitative applications of this method of separation, especially with reference to the bile salt fraction, will be reported shortly.

METHOD

1. *Reagents.*—

1. 0.5N potassium hydroxide.
2. Ten per cent zinc sulphate ($ZnSO_4 \cdot 7H_2O$).

This is the concentration of alkali and zinc sulphate recommended by Somogyi[18] for the deproteinization of blood. Potassium hydroxide has been substituted for the sodium hydroxide since its carbonates and sulphates crystallize with little or no water of crystallization. The two solutions are related, however, as recommended, so that 10 c.c. of the zinc sulphate are neutralized to phenolphthalein by 10.8 or 11.2 c.c. of the alkali.

3. Ninety-five per cent and absolute ethyl alcohol.
4. Petroleum ether, boiling point 30-80° C.
5. Approximately 4N (22.4 per cent) potassium hydroxide.
6. Approximately 2N (14 per cent) potassium carbonate (K_2CO_3).
7. Approximately 5N sulphuric acid, 139 c.c. concentrated sulphuric acid per liter.
8. Six per cent potassium hydrogen phosphate (K_2HPO_4).

2. *Procedure.*—The quantity of bile required depends upon the constituent being determined. It has been found convenient to use such an aliquot of bile as will provide at least 25 mg. of bile salts or 0.5 mg. of cholesterol. This is usually achieved by using 1.0 c.c. or less of gallbladder bile or from 2.0 to 4.0 c.c. of fistula bile.

The quantity of bile must be carefully measured, preferably using a "to contain" pipette, into a 50 c.c. centrifuge tube. The pipette is rinsed two or three times with distilled water, using sufficient to bring the total volume to 10 c.c. Add 1 c.c. of the 0.5N potassium hydroxide, then add 1 c.c. of the zinc sulphate with gentle mixing. The contents of the tube are thoroughly mixed to obtain maximum adsorption of the pigment and precipitation of the protein. The tube is centrifuged for about five minutes. If the supernatant is not water clear, the addition of a few drops of the alkali followed by a similar amount of the zinc sulphate, will complete the adsorption. It is advisable to loosen any particles of the green precipitate from the upper portion of the tube by means of a fine stirring rod before the final centrifuging. The water-clear supernatant is decanted off into an Erlenmeyer flask.

To the precipitate is added about 9 c.c. of distilled water and the flocculent precipitate is stirred up thoroughly with a stirring rod which is rinsed off with about 1 or 2 c.c. of water. The tube is centrifuged and decanted off as before. This aqueous extract is added to the original supernatant. A second similar extraction is made with distilled water. These aqueous extracts remove not only a portion of the bile salts but also the excess zinc which should be removed by precipitation. Add 1 c.c. of 2N potassium carbonate to the combined aqueous extracts, mix and filter through a fat-free filter paper into a 125 c.c. Erlenmeyer flask. Wash the precipitate 2 or 3 times with small portions of distilled water to remove any bile salts.

The remaining portion of the bile salts and the cholesterol are removed by at least five similar extractions with 95 per cent ethyl alcohol. The first two are at room temperature while for the others the tube is placed in a water-bath at 60 or 70° C. for about five minutes with occasional shaking to prevent the precipitate from settling out. All of these alcoholic extracts are combined with the zinc-free filtrate forming the bile acid-cholesterol fraction.

The separation of the cholesterol from the bile salts presents little difficulty. Add 5 c.c. of 4N KOH to the bile salt-cholesterol extracts and evaporate to about 20 c.c. on the steam bath. This requires about thirty minutes which is sufficient to hydrolyze the cholesterol

esters but not the conjugated bile salts. At the end of this time 5N sulphuric acid is added to the first permanent turbidity due to the precipitation of free bile acids. The solution is then cleared by the gradual addition of 2N K_2CO_3, 2 c.c. in excess being added. Evaporate to dryness on the steam bath. The last traces of moisture may be removed by drying at 110° C. for not over thirty minutes. At least three extractions with 10 or 15 c.c. of petroleum ether, boiling up each time, and filtering from any solid particles if necessary are required. The petroleum ether extracts may be evaporated to dryness, taken up in chloroform and the cholesterol determined in the usual manner.

Bile salts may be separated from the potassium sulphate and carbonate by at least 5 extractions with absolute ethyl alcohol as above. The bile salts may be then estimated by some suitable procedure. A method to be published shortly has been developed to estimate the bile salts in this fraction.

The pigments may be readily removed from the precipitate. Add about 5 drops of 0.5N KOH and 2 c.c. of 6 per cent K_2HPO_4 to the precipitate in the centrifuge tube. Stir thoroughly with a stirring rod in order to dissolve the pigment and to precipitate the zinc as the phosphate. Water or 95 per cent alcohol is then added slowly and with stirring to a volume of about 10 c.c. Place the tube in a hot water-bath for about five minutes with frequent stirring to favor solution. After centrifuging a clear green solution and a pale precipitate will be obtained. A second extraction with alcohol or water will remove the last trace of pigment.

SUMMARY

A simple method has been developed to separate the bile pigments, bile salts and cholesterol from the same specimen of bile. Each of these fractions may then be estimated without the interference of other constituents.

The author wishes to express his appreciation to Dr. J. B. Collip and Dr. D. L. Thomson of the Department of Biochemistry for their many helpful suggestions and criticism.

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A MODIFICATION OF THE LANGE COLLOIDAL GOLD TEST*

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SINCE the introduction of the colloidal gold test by Lange in 1912 many modifications have been reported. Nearly all of these changes have been in connection with the preparation of the reagent while the actual performance of the test has remained practically unchanged. The difficulties encountered in the preparation of suitable gold solutions accounts for the interest shown in this particular phase of the test. At best the preparation of the reagent is tedious, time consuming, and the solutions often fail to meet the necessary requirements of sensitivity.

We found that, by changing the method of diluting the spinal fluid, the test could be conducted with one-half the amount of reagent. This results in a saving of time and material as twice the number of tests can be performed with the same amount of reagent.

A series of parallel tests were conducted on each spinal fluid using the original method with 5 c.c. of reagent and the modification outlined below using 2.5 c.c. of reagent. A total of 12 fluids were tested, of which 5 gave typical Zone I (paretic) curves, 4 gave typical Zone II (syphilitic) curves, and 3 were negative. The reactions in the modified test were practically identical with those in the original test. A slight difference in the degree of reduction in a few of the corresponding tubes of each test was noted. This difference when present was at the beginning or end of the curves and not sufficient to alter the readings. Neither test proved to be more or less sensitive than the other.

To obtain additional proof that the amount of reagent used in the test could be varied, providing the fluid tested remained in the proportion, several lots of reagent were titrated for sensitivity by the method recommended by Ramsey and Eilmann.† In each case five separate titrations were conducted using 5, 4, 3, 2, and 1 c.c. of reagent with a corresponding reduction in the amounts of sodium chloride solution. The amount of reduction or sensitivity titer was exactly the same in all set-ups. This shows that the test can be conducted with any amount of reagent providing the test fluid is used in the same proportion as recommended in the original test. We feel, however, that the

*From the Laboratories of the Graduate Hospital, University of Pennsylvania.
Received for publication, September 21, 1933.

†Ramsey, Thomas L., and Eilmann, H. J.: The Preparation of Colloidal Gold Solution, J. Lab. & Clin. Med. **18**: 298, 1932.

amounts recommended below are more practical than smaller amounts, due chiefly to the possible introduction of error when dilutions are made with less than one-tenth cubic centimeter of spinal fluid.

Although the results obtained in these tests would be expected, as there was no logical reason to the contrary, yet one is reluctant to modify in the least a well-established technic of such an important test without first obtaining proof that such a change would not in some way interfere with its accuracy.

We have since used the modified method outlined below routinely with very satisfactory results.

Modified Colloidal Gold Test.—

1. Place eleven chemically clean test tubes in a rack.

2. Into the first tube place 1.8 c.c. of 0.4 per cent sodium chloride solution and 0.5 c.c. in each of the remaining 10 tubes.

3. Add 0.2 c.c. of spinal fluid to the first tube and thoroughly mix. (We feel that the dilutions will be more accurate if 0.2 c.c. of fluid is diluted with 1.8 c.c. of salt solution rather than 0.1 c.c. of fluid with 0.9 c.c. of salt solution. If the latter is used, the 1 c.c. is not discarded from the first as directed in Step 4.)

4. Discard 1 c.c. from the first tube, then transfer 0.5 c.c. to Tube 2; mix thoroughly and remove 0.5 c.c. and place in Tube 3; continue until the tenth tube is reached and then discard 0.5 c.c. from this tube. The eleventh tube is used as a control.

5. Add to each tube 2.5 c.c. of colloidal gold solution.

6. Mix thoroughly and set aside for twenty-four hours. The readings are now made and recorded the same as in the original test.

CONCLUSION

1. The Lange colloidal gold test can be conducted with varying amounts of reagent providing the proportion of spinal fluid remains the same.

2. A modification of the test is recommended which requires but one-half the amount of reagent that is used in the original method.

A DROPPER FOR PERFORMANCE OF FRAGILITY TEST OF RED BLOOD CORPUSCLES*

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IN THE performance of the red blood cell fragility test much difficulty was experienced in obtaining the desired dilutions of half percentage saline and distilled water when using the ordinary pipette for counting the number of drops.

To prepare the different strengths of saline solution by the drop method requires that the count of the number of drops of saline delivered to each test

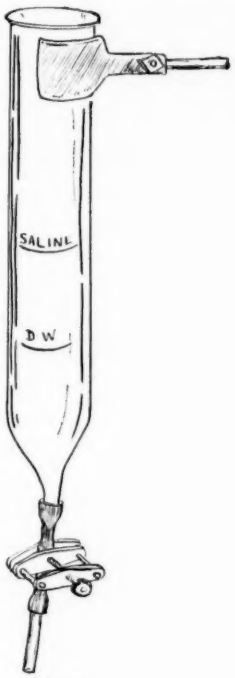

Fig. 1.

tube be absolutely accurate and that all drops be equal in size. The use of the apparatus to be described has decreased the time necessary for performance of the test, has simplified the mechanical manipulations, and has increased the dependability of the results.

A large test tube (1 inch in diameter and 6 inches long) is heated in the Bunsen flame and pulled until the cross-section at the narrowest part is about one-eighth of an inch. The glass is cut at the desired point so that a two-inch section of small rubber tubing like that used in blood-counting pipettes may be

*From the Temple University Hospital.
Received for publication, September 5, 1933.

pulled over it and firmly held by its own elasticity. A short section of glass tubing (about 1 mm. inside diameter) is attached to the lower end of the rubber tubing and an adjustable screw clamp is placed on the middle of the rubber tubing, completing the apparatus.

When performing the test, the apparatus is fastened in a ringstand and filled with the solution to be measured. The clamp is loosened until a drop of fluid forms on the tip of the glass nozzle every eight or ten seconds. If now the lower end of the rubber tube and the glass tip be pulled downward, putting the rubber tube on the stretch, the rate at which the fluid runs through the tube will increase roughly proportional to the amount of tension on the hose, the solution falling regularly from the glass tip in drops of uniform size. Since the tension on the rubber tubing is so easily controlled with one hand and the flow is so uniform, the drops can be very easily counted and the flow instantly stopped at the desired moment. Meanwhile the other hand remains free to slide the test tube rack and its tubes consecutively under the dropper until each tube has received its predetermined number of drops of liquid. Saline and distilled water may be measured through the same apparatus with only a rinsing between.

A few trials will show the operator just how much saline and how much distilled water is necessary for the test, so that file marks may be placed at the proper levels on the large test tube as a guide in conserving these substances. When not in use the apparatus should be rinsed, the clamp loosened, and the whole laid away for future use.

3401 North Broad Street

SEDIMENTATION TIME OF BLOOD*

Improved Apparatus for Routine or Research Tests

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THE expense of the graduated test tubes usually employed in determining the sedimentation rate of blood has been saved by the use of the apparatus described, which uses ungraduated test tubes which are read against a graduated background. The technic of the test remains essentially the same as with graduated tubes.

The apparatus consists of two rectangular pieces of wood or other substance so fastened together as to form a baseboard upon which the test tubes stand and a backboard which supports the tubes and which bears a millimeter scale easily drawn with the aid of a straight edge and millimeter rule. One horizontal line is drawn 51 mm. above the plane upon which the test tubes rest, i.e., the baseboard. This line, which is marked "0," corresponds to the level to which the blood citrate solution appears in the tube when properly filled. The other mark-

*From the Temple University Hospital.
Received for publication, September 5, 1933.

ings are made parallel to this "0" line at 10 mm. intervals. Finer lines may be drawn at 5 mm. intervals if desired. The completed scale (see Fig. 1) is used for determining the level between the cells and plasma in the tubes, which may be read directly to 5 mm. and to 1 mm. by interpolating between the nearest lines of the scale. Horizontal lines at millimeter intervals are easily ruled, but to us seemed unnecessary and confusing. The scale is most easily drawn on white paper with waterproof ink, carefully fastened to the backboard in the desired position and then covered with a piece of transparent celluloid film such as is used as a base in photography and radiography.

The test tubes are conveniently held in place with a continuous piece of soft brass wire which will not rust, become loose, or pull out. The lower row of wire loops should be placed about 7 mm. from the baseboard, instead of in the

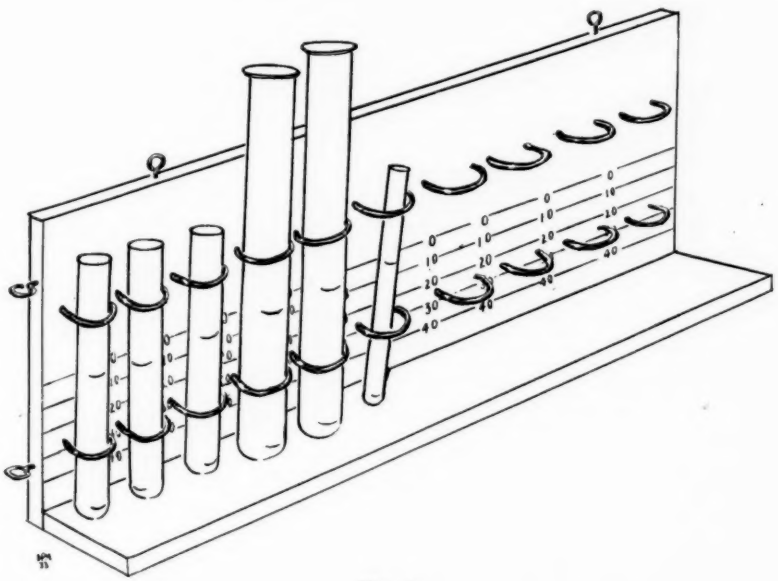

Fig. 1.

position shown in the illustration. When in the lower position they do not interfere with reading the scale, yet securely hold the tubes—the first requisite of any test tube rack. As shown, this rack accommodates tubes having an inside diameter of 15 mm., or any tube of smaller caliber. This feature enables one easily to study the effect of tube diameters upon the rate of fall without the necessity of graduating the tubes themselves. A set of eyes in the upper edge of the backboard will hold the rack at eye level when fastened to hooks or nails at the proper height on the wall. A set of eyes on the side is useful in swinging the rack from vertical fixtures such as pipes or the corner of a projecting shelf where the rack would be placed more conveniently than if it were against the wall. Since the tubes are against the scale against which they are read and the rack is at eye level, there is a negligible error in making readings in this way. While the rack was made of quarter inch pine board, I should think Bakelite would be the ideal substance.

In our sedimentation tests the 11 mm. tubes were used for routine, although larger and smaller tubes were used experimentally. Regardless of the size of the tubes, they may be prepared in the same way. With a millimeter rule, a single file mark is made 7 mm. from the base upon which the tubes rest and another one 51 mm. from the base. The file mark is easily filled with colored crayon or India ink and the excess wiped off, leaving a mark clearly visible.

In use, 2 per cent sodium citrate is dropped into the tube until the meniscus reaches the lower mark and blood is added until the meniscus of the mixture reaches the upper mark. The amount of citrate should be carefully measured because any appreciable variation from this standard amount will change the rate of fall to a greater extent than will any of the other technical procedures.

3401 North Broad Street

NOTE ON THE PROCEDURE IN THE KUTTNER-LICHTENSTEIN MICROCOLORIMETRIC METHOD FOR THE DETERMINATION OF ORGANIC PHOSPHORUS*

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THE microcolorimetric method, developed by Kuttner and Lichtenstein[1] for the determination of organic phosphorus, involves digestion with sulphuric acid, aided by 30 per cent hydrogen peroxide of which the excess must be removed, and addition of sodium molybdate and stannous chloride to form a blue color. The only uncertain and tedious step in the procedure is the removal of excess hydrogen peroxide.

In the original method this was accomplished by adding water to the cleared digestion mixture, boiling down, adding more water, and repeating the process a number of times. From two to four hours are required to decompose the hydrogen peroxide completely, and almost constant attention is necessary. An alternative method, requiring little attention, consists of keeping the tubes in a hot water-bath for two or three days, and replacing the evaporated water from time to time.

The present modification eliminates both the tedious and time-consuming factors. It is carried out in a simple way as follows:

After the digestion mixture has been finally cleared (for some organic materials this may take a few additions of hydrogen peroxide with subsequent heating after each), it is digested for three minutes after the first white fumes appear. Care should be taken not to lose sulphuric acid by excessive fuming, since it has been shown[2] that, if the acid concentration in the final blue solution is less than 0.9 N, additional color is produced. Accordingly, the flame should be regulated so that white fumes appear in the bottom of the tube, but condense farther up.

*From the Laboratories of The Mount Sinai Hospital.
Received for publication, September 19, 1933.

At the temperature of the boiling concentrated sulphuric acid, the three-minute period is sufficient to decompose the excess hydrogen peroxide. The excessive time required in the original procedure is the result of the relative stability of hydrogen peroxide at the boiling temperature of the dilute acid.

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A SIMPLE TECHNIC FOR FINDING COCCIDIOIDES IMMITIS*

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RECENTLY, while searching fresh moist preparations of sputum from a case of coccidioidal granuloma, for the characteristic spherical bodies, I found that they showed a strong iodine staining power. If enough Lugol's or Gram's iodine solution be mixed with the sputum or sinus tract pus to

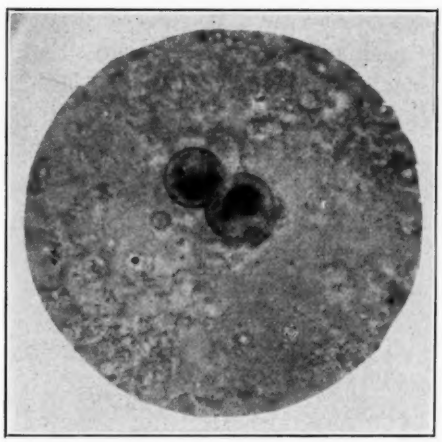

Fig. 1.—Coccidioides immitis, iodine stain (×640).

color the other cells a lemon yellow color, the coccidioidal bodies will take a rich brown tint within five minutes and can be located easily by the low power objective.

Having seen no reference to this simple trick, I offer it to those who have occasion to look for these organisms. A large amount of material can be searched very quickly, and fat droplets or air bubbles that are confusing, at least to those unfamiliar with the organism, can be quickly excluded.

Fig. 1 shows the strong contrast produced.

*From the Department of Pathology, St. Luke's Hospital.
Received for publication, September 22, 1933.

THE ESTIMATION OF ETHYL ALCOHOL IN BRAIN*

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I. ALCOHOL IN EMBALMED TISSUES

THE identification and quantitative estimation of ethyl alcohol in the embalmed body has heretofore been difficult, if not impossible. Yet tissues from embalmed bodies are often sent to the chemist with the statement that alcoholism is the probable cause of death, and the chemist is expected to establish the point. We, therefore, planned experiments in the hope of developing a dependable quantitative method, based upon the method of Gettler and Tiber.[1]

Briefly, the Gettler and Tiber method consists of a steam distillation of a weighed portion of the brain (500 gm.); an aliquot portion of the steam distillate is then treated with potassium dichromate and sulphuric acid, redistilled, and an aliquot of the resulting acetic acid distillate titrated against twentieth normal sodium hydroxide. The percentage of ethanol is then calculated in terms of the sodium hydroxide titration, 1 c.c. of twentieth normal sodium hydroxide being equivalent to 2.3 mg. ethanol.

Throughout, the procedure of Gettler and Tiber has been followed, except where modification has been necessary to accommodate the amount of tissue used, and where the purpose of the experiment required. Preliminary to the investigation of tissue, standard mixtures of formaldehyde, methanol, and ethanol were used. The concentrations of ethanol in these mixtures were approximately those found in brain tissue, viz., from 0.4 per cent to 0.6 per cent. The method of removal of methanol and formaldehyde is based on the ease of oxidation of these compounds to carbon dioxide and water. Acetic acid, the oxidation product of ethanol, is extremely stable.

Direct distillation of the mixture from dichromate and sulphuric acid was first tried. On titration the results were quite variable, and in most instances, the values were much higher than was warranted by the amount of ethyl alcohol present. It was assumed that the increased acidity above that for acetic acid was due to formic acid, and no qualitative estimation of the oxidation products was undertaken. This experiment was repeated with the introduction of a preliminary period of refluxing before distillation. Upon distillation and titration the results were in agreement with the amounts of ethanol present in the original mixtures. This was accounted for by the fact that in refluxing, the methanol and formaldehyde are oxidized to carbon

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Received for publication, September 22, 1933.
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dioxide and water, while the oxidation product of ethanol, namely acetic acid, is stable in the oxidizing mixture. The above results were duplicated many times.

The time of refluxing was varied. It was found that from thirty to forty-five minutes at a slow boiling point was sufficient for complete oxidation.

The above procedure was then applied to brain tissue of rabbits. The animals ranged from 1.5 to 3.3 kilograms in weight.

Five rabbits were used for an estimation of a possible normal alcohol content of the brain. They were housed for a period of one week prior to use, and fed upon carrots, lettuce, and whole bran.

The animals were killed by the neck stroke, and the brain removed, placed in an agate mortar, and thoroughly macerated. The macerated tissue was then washed into a 2 liter flask to which were added 300 c.c. of distilled water, a few drops of mineral oil and 1 c.c. of saturated tartaric acid solution. The flask was then connected to a steam still fitted with a Cummings bulb, and 300 c.c. of distillate was recovered. The entire steam distillate was placed into a 500 c.c. flask to which 20 gm. potassium dichromate and 40 c.c. of concentrated sulphuric acid were added. The flask was connected to an upright spiral condenser and 250 c.c. of distillate recovered. One hundred cubic centimeters of this distillate were titrated against N/100 NaOH. The titrations never exceeded 2.5 c.c. of the standard solution. An aqueous acid control distillation carried out in the same manner gave the same titration figures. Due to the agreement in blank and titration of brain distillates, it was assumed for the purpose of the experiment that the brains were negative for alcohol.

A second group of 5 rabbits were given by stomach tube, ethyl alcohol at a dosage of 6 gm. of ethanol per kilogram weight. The alcohol solution was of the strength of average alcoholic beverages (brandy, 50 per cent). The animals were killed, at one, two, five, ten, and twenty-four hours after the alcohol administration and the brains analyzed for alcohol, to determine roughly the time for development of maximum alcohol concentration in the brain. Table I shows the results of the experiment.

A second group of 13 rabbits were given the alcohol solution in the same dosage (6 mg. per kg.). Five animals were killed after an interval of five hours and the ethanol content of the brain estimated immediately; 8 animals were killed and embalmed immediately, and the brain removed for analysis one hour later. Two more animals received half the above dosage (3 mg. per kg.) and were killed five hours later for analysis of their brains. Finally,

TABLE I

RABBIT	TIME ELAPSED	PER CENT ETHANOL
4	1 hour	0.321
10	2 hours	0.325
11	5 hours	0.359
10a	10 hours	0.278
12	24 hours	0.0031

three animals received this latter dosage, were killed after five hours and were embalmed one hour prior to removal of the brain. Table II shows the results of the experiment.

A control group of 5 animals were then killed in the same manner and embalmed. Upon analysis of the brain tissue the results were in accordance with those which were determined upon fresh brain tissue of control rabbits, the figures checking closely with blank titrations.

SUMMARY AND CONCLUSIONS

By modification of the Gettler and Tiber technic, introducing a period of refluxing of the dichromate-sulphuric acid step in the procedure, formaldehyde and methanol may be completely removed, so that only the ethyl alcohol fraction, namely acetic acid, appears in the final distillate for titration. Rabbits were used for the experiments. It seems safe to conclude that by this method, quantities of alcohol of medicolegal importance can be determined in brains of embalmed rabbits, and probably of embalmed human brains.

II. A MODIFICATION OF THE GETTLER AND TIBER METHOD FOR TRACES OF ALCOHOL

In the routine analysis of human brains for alcohol in the laboratory of the Los Angeles County Health Department, wherein the method of Gettler and Tiber was employed, more than 100 brains were tested whose titration figure was 0.9 c.c. or less of 0.05N NaOH. Such brains were reported to the coroner as negative for alcohol. Recalling, however, that Gettler and Tiber found an average of 0.0015 per cent alcohol in normal brains by correcting for organic acids in blanks similarly obtained, an effort was made to modify the method to reduce the total titration instead of correcting for it. The first

TABLE II

UNEMBALMED BRAIN 6 GM. ALCOHOL PER KG.		EMBALMED BRAIN 6 GM. ALCOHOL PER KG.	
NO.	PER CENT ETHANOL	NO.	PER CENT ETHANOL
20	0.36	A.	0.36
22	0.41	B.	0.28
23	0.38	D.	0.21
24	0.41	E.	0.52
25	0.45	F.	0.38
		26	0.32
		27	0.44
		28	0.24
3 GM. PER KG.		3 GM. PER KG.	
29	0.15	31	0.16
30	0.15	32	0.14
		33	0.078

distillate from the brain contains alcohol and certain titrable organic compounds; the second distillate contains similar titrable substances in addition to the acetic acid, resulting from the oxidation of the alcohol. Gettler and Tiber calculated the normal alcohol present by subtracting the acidity of the first distillate from the acidity of the second distillate. The present study was based upon the supposition that the insertion of an alkaline distillation between

the first and second regular distillations would hold back the interfering materials. The procedure was as follows:

Five hundred grams of tissue was steam distilled to collect 800 c.c. After filtration, 300 c.c. was treated with 20 grams of potassium dichromate and 40 c.c. of sulphuric acid and distilled to collect 250 c.c. Fifty c.c. was titrated for the report to the coroner. When the titration figure for the 50 c.c. was 0.9 c.c. 0.05N NaOH, or less, the remainder of the steam distillate (500 c.c.) was treated with 50 c.c. of 3N NaOH or the equivalent amount of Ca $(OH)_2$ for the purpose of forming non-volatile soaps of contaminating organic acids. This mixture was steam distilled to collect 500 c.c., 300 c.c. was treated with dichromate and sulphuric acid and distilled to collect 250 c.c. and the entire distillate titrated. The figure for the reagent blank of 0.65 c.c. 0.05N NaOH was subtracted. One c.c. 0.05N NaOH is equivalent to 0.0013 per cent alcohol. This factor includes the correction of 90 per cent recovery in the acid distillation besides the corrections of dilution and conversion of acetic acid to alcohol.

Distillations from seven brains have been tested by the above procedure, with results as indicated in table III.

Before further work on the problem was undertaken, the paper by Gettler, Niederl, and Benedetti-Pichler[2] appeared, showing the average alcohol content of seven brains to be 0.0004 per cent, with a range of 0.00027 to 0.00055 per

TABLE III

TREATED WITH NaOH				TREATED WITH $Ca(OH)_2$			
NO.	FIRST ACID DISTILLATE 0.05 N NaOH C.C.*	SECOND ACID DISTILLATE 0.05 N NaOH C.C.	ALCOHOL PER CENT	NO.	FIRST ACID DISTILLATE 0.05 N NaOH C.C.*	SECOND ACID DISTILLATE 0.05 N NaOH C.C.	ALCOHOL PER CENT
Blanks		0.65		Blanks		0.65	
2940	3.5	1.6	0.0013	3006	4.5	1.0	0.0005
2959	5.0	1.3	0.0009	3007	4.0	1.0	0.0005
2968	3.5	1.4	0.0010	3008	4.0	0.7	
				3012	3.0	0.7	

*Calculated for 250 c.c. of distillate.

cent. It will be seen that when $Ca(OH)_2$ was used to prevent volatilization of organic acids in the steam distillation process, the alcohol values for two brains by the titration method approached the values obtained by Gettler, Niederl and Benedetti-Pichler, using much more refined technic. The titration figures for two other brains by the $Ca(OH)_2$ method were so close to the blanks that calculations of percentage would have had no significance.

It is suggested that the figures for the $Ca(OH)_2$ distillation are lower than those for the NaOH distillation because of the lower volatility of the resulting calcium compounds.

Because present circumstances prevent further investigation of this type, and because of the closeness of the figures obtained by the modified distillation process with those by the more difficult procedure of Gettler and his

associates, the present results with the small number of brains are reported. It is believed that the method is useful for the determination of minute amounts of alcohol in human tissue.

The authors wish to acknowledge the helpful guidance and encouragement of Dr. R. V. Stone, Director of the Bureau of Laboratories, Los Angeles County Health Department.

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THE DIAZO REACTION FOR DETECTION OF CERTAIN LOCAL ANESTHETICS IN URINE AND IN TISSUES*

W. E. Gibb, B.S., M.S., and William M. Dehn, Ph.D., Seattle, Wash.

EGGLESTON and Hatcher[1] showed that procaine is rapidly destroyed by the liver. Thieulin[2] found that both it and its hydrolytic products were eliminated in the urine of animals. We have found that it can easily be detected in the urine a few minutes after injection in the tissues by application of Riegel's and Williams'[3] modification of the diazo reaction. In its most simple form one needs only to add in order a little sodium nitrite, acetic acid, and ammonia to observe the red or yellow formed, and compare the color with that of untreated urine. Any change of color is proof of the presence of procaine, etc., or their hydrolytic products such as meta or p-aminobenzoic acids, which because of contained amino groups also give colors with the reagent.

In a cadaver known to contain procaine, comminuted tissues made acid in reaction and then extracted with ether gave colors identical with the colors produced with the extracts from tissues made alkaline in reaction. This observation proves that procaine is hydrolyzed in the body, either before or after death. For this reason accurate quantitative analyses of tissues for procaine must involve the separation of procaine and its hydrolytic product, p-aminobenzoic acid.

In Table I are recorded data on the diazo reaction as applied to 1,233 specimens of urine examined in a hospital over a period of three months. All these patients had received medicine as administered in hospital practice, and only about 5 per cent of them had received procaine.

Seven of the patients showing apparent positive test for procaine, but not receiving procaine, were found to have gallbladder disease; these urines gave positive Van den Bergh tests for serum bilirubin. The other two patients did not return for further investigation. When apparent positive tests were indicated, the colors were not as distinct as in the tests following administration of procaine.

*From Virginia Mason Hospital, and Chemistry Laboratory, University of Washington. Received for publication, June 21, 1933.

TABLE I

NUMBER PATIENTS RECEIVING PROCAINE

	TOTAL	POSITIVE AFTER			NEGATIVE
		1 HR.	2 HR.	3 HR.	
½%, 30 c.c.	17	14	2	1	0
¾%, 30 c.c.	2	1	0	0	1
1%, 6-12 c.c.	39	38	1	0	0
4%, 12 c.c.	2	2	0	0	0
2%, 2 c.c.	4	4	0	0	0

	TOTAL NUMBER EXAMINED
Urine examined	1,233
Receiving procaine	64
Positive first hour	59
Positive second hour	3
Positive third hour	1
Procaine negative (urine lost)	1
Not receiving procaine but giving apparent positive reaction	9
Negative urine	1,160

In all cases not showing a positive test during the first three hours there was evidence of impaired kidney function with some retention of urine. Three of these patients had a high blood urea and creatinine. Morning urine, which showed a high degree of color, showed no reaction or change in color in testing for procaine. As yet no specimens of urine except the seven mentioned above have given a test for procaine, except where procaine was given.

SUMMARY

Riegel's and Williams' test is a quick and reliable test for procaine and other aminobenzoic acid derivatives used as local anesthetics, when excreted in the urine or when present in the cadaver. Over 1,100 specimens of urine of patients not receiving procaine gave negative tests, except that a few cases gave apparent test when gallbladder diseases were present. Hydrolytic products of procaine were found in the tissues of a cadaver.

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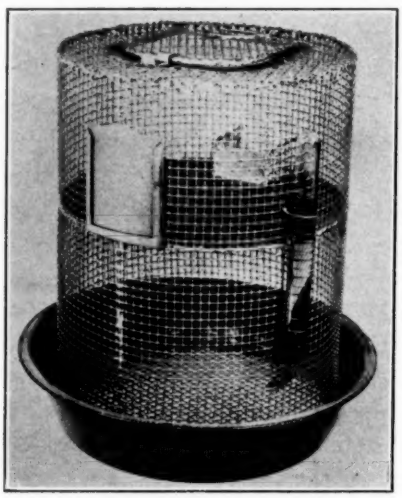
2. Thieulin: J. de pharm. et chim. **22**: 463, 1920.
3. Riegel and Williams: J. Am. Chem. Soc. **48**: 2871, 1926.

CAGE FOR MICE AND RATS*

ALLAN W. BLAIR, AND EMMETT B. CARMICHAEL, UNIVERSITY, ALA.

THIS type of cage has been used by us for the past two years, and we feel that it possesses distinct advantages over other types of cages used heretofore. In our experience some of these advantages consist in the prevention of food and bedding pollution by the segregation of each in a different compartment, the ease and cheapness of construction, the greater accessibility of the animals, the ease of cleaning, the ease of observation of the undisturbed animal and the ease of transportation from place to place. The cage serves well for feeding experiments, breeding purposes, stock animals, and general experimental purposes. It is compact, economical of space, and may be



placed on a narrow shelf. Waste cotton is employed for bedding and it is placed in the upper compartment. If so desired, the food may be placed in the upper rather than the lower compartment.

Dimensions.—11.25 x 8.5 inches.

Materials.—Galvanized hardware cloth (1/4 inch mesh) for body, door, removable floor, and as a guard around opening between the two compartments (to prevent bedding material from falling through into lower compartment); sheet zinc (1/32 inch) for floor of upper compartment, card holder, and to form margin of door opening; sheet metal (1/16 inch) for band at bottom of cage and door catch; heavy wire soldered to edge of mesh door (to provide additional strength and support); water container (6 inch test tube, single hole rubber stopper, glass tubing) fastened by detachable clips to side of body; enamel pan.

*From the School of Medicine, University of Alabama.
Received for publication, August 2, 1933.
This article is being republished from the May issue because of the omission of the illustration.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DIPHTHERIA, Precipitated Toxoid as an Immunizing Agent Against, Baker, J. N., and Gill, D. G. Am. J. Pub. Health 24: 22, 1934.

A single injection of 1 c.c. of precipitated toxoid has rendered 100 per cent of 197 Schick-positive children Schick-negative. Similarly, 1,400 of 1,414 children, or 99 per cent, were Schick-negative when tested on an average of from two to three months after a single injection. The original immunity status was unknown. Observations on 16,289 inoculations revealed 8 with abscess formation. As a rule local or general reactions were not severe.

ERYSIPELOID, Among Workers in a Bone Button Factory Due to the Bacillus of Swine Erysipelas, Mc Ginnes, G. F., and Spindle, F. Am. J. Pub. Health 24: 32, 1934.

Two hundred and ten cases of an erysipeloid condition occurred in a bone button factory among individuals handling cattle or hog bones. There was a close correlation with the handling of wet bone and occupations in which injuries were frequent. Repeated attacks of the disease were frequent, indicating that there was little or no local or general immunity produced by an attack. The bacillus swine erysipelas was isolated from one case and from samples of bone, bone material, and bone waste collected at various stages in the manufacture of buttons. For ridding the plant of infection the heating of all bones entering the plant in the soaking vats to a temperature of 144° F. for at least two hours is recommended.

TUBERCULOSIS, Blood Culture in, Penfold, W. J., and Butler, H. M. Med. J. Australia 2: 837, 1933.

In three out of twenty cases of tuberculosis it was possible to cultivate Bacillus tuberculosis from the blood by Lowenstein's method. In the cultures from one other case acid-fast organisms could be identified microscopically by means of smears made from the surface of the medium; but these cultures, though incubated for six months, failed to produce colonies visible to the naked eye. Subcultures from them likewise failed to grow.

The Lowenstein method of blood culture in tuberculosis is troublesome and time-consuming in execution and gives so few positive results that the authors cannot believe that it is destined to render any great service to the clinician in the diagnosis of tuberculosis.

DIABETES, Insulin Resistance in, Mac Bryde, C. M. Arch. Int. Med. 52: 932, 1933.

Attention is called to the frequency of occurrence of relative resistance to insulin in uncomplicated cases of diabetes mellitus.

Clinical studies of two cases of insulin-sensitive and three cases of insulin-resistant diabetes are reported.

In two of these patients resistance to endogenous insulin was apparently broken, an overdosage of insulin being followed by marked improvement in carbohydrate tolerance.

The clinical characteristics of the insulin-resistant type as distinguished from the insulin-sensitive type are outlined.

Reported cases of insulin resistance may be grouped as follows:

(a) Unexplained, such as the five cases reported in this paper. Insulin resistance is

usually of mild or moderate degree in this group. Hyperactivity of the suprarenal-sympathetic system is suggested as the cause of this type of resistance. Many of these patients might be termed constitutionally resistant.

(b) Infection.

(c) Destructive pancreatic disease.

(d) Disorders of other endocrine glands, particularly the thyroid, pituitary and suprarenal glands.

(e) Hepatic disorders.

(f) Miscellaneous conditions such as acidosis, coma, diseases of the skin and cardiac decompensation.

The value of determining the relative responsiveness to insulin for treatment as well as for further study of diabetes is emphasized.

MYELOSIS, Aleukemic, Baldridge, C. W., and Fowler, W. M. Arch. Int. Med. **52:** 852, 1933.

Ten cases are reported in which a common feature was myeloid hyperplasia.

Two cases were ordinary instances of leucemic myelosis, except that in the course of each there developed an almost completely aleucemic period. In one case the aleucemic period was thought to be due to irradiation of the spleen; in the other, it was "spontaneous."

Four cases thought to be diffuse hyperplasia of the myeloid tissue were characterized by severe anemia, leucopenia, a few abnormal leucocytes, secondary hemorrhagic purpura, changes in the bones and extramedullary collections of myeloid cells.

Two cases were indistinguishable from ordinary multiple myeloma, except that the tumors were made up of myeloid cells instead of plasma cells. One case presented chemical changes sometimes seen in multiple myeloma of the plasma cell type.

In the last two cases extramedullary myeloid tumors were present without definite evidence of disease of the bone marrow.

ANEMIA, Macrocytic in Disease of Liver, Van Duyn, J. 2nd. Arch. Int. Med. **52:** 839, 1933.

An introductory case illustrating the occurrence of macrocytic anemia in cirrhosis of the liver is briefly reported.

References from the literature show this to be a widely recognized syndrome.

Three cases of disease of the liver are reported in which personal study has confirmed the macrocytic character of the anemia.

Twenty-eight cases of hepatic cirrhosis were reviewed from the records of the Syracuse University Hospital. Macrocytic anemia was found in 5 cases, or 18 per cent.

The mechanism producing a macrocytic type of anemia is discussed. Diseases of the liver is given a place in this mechanism.

SCHULTZ-CHARLTON TEST, Fischer, A. E., and Kojis, F. G. Am. J. Dis. Child. **46:** 1282, 1933.

From a study of this reaction as well as the calcium blanching test in 486 cases the authors conclude that:

The Schultz-Charlton test is specific for the rash of scarlet fever.

A 10 per cent solution of calcium gluconate will cause slight blanching in some early cases of scarlet fever in which a mild or moderately intense rash is present. This action, however, is not so constant or so persistent as that obtained with dilute scarlet fever antitoxin.

The actions of calcium and of antitoxin on the rash of scarlet fever are totally different.

The fact that calcium will blanch a rash does not in any way detract from the validity of the Schultz-Charlton test.

The Schultz-Charlton test, provided its limitations are understood, is invaluable in the early diagnosis of scarlet fever.

MALARIA, A New Antigen for Henry's Reaction, Livierato, S., Vagliano, M., and Constantacato, G. Greece Med. **31**: 177, 1933.

The sac of a cuttlefish with its contents is cut up into small pieces and put into a sterile tube; 40 c.c. of sterile distilled water are added, and 0.02 c.c. of formalin. After twenty-four hours at room temperature, the supernatant fluid is pipetted off; it is kept in the ice chest for two months before use, and is shaken daily. It is diluted to make a slightly blackish fluid just before use. The results are the same as those obtained with Henry's ox-eye melanin.

SYPHILIS, Blood Cytology in Treated and Untreated, Rosahn, P. D., and Pearce, L. Am. J. M. Sc. **187**: 88, 1934.

From a study of the blood cytology of 126 treated and 87 untreated syphilis patients it was found that:

Treatment causes a fall in the total white cell count, the platelet count, and the relative and absolute numbers of neutrophiles and monocytes, and a rise in the relative and absolute numbers of lymphocytes, and per cent hemoglobin, from values observed in the untreated cases.

No significant differences were noted between the mean blood cell values of the primary and secondary untreated patients.

A marked anemia was the outstanding feature of the blood cytology in the untreated tertiary disease. This anemia was absent in patients whose treatment was begun in the tertiary stage.

No differences in the blood cytology of treated patients were noted when treatment had been begun in the primary, secondary or tertiary stages of the disease.

A monocyte-lymphocyte ratio higher than 0.55 was more frequent among untreated patients and a monocyte-lymphocyte ratio less than 0.55 was more frequent among treated patients than would be expected from random association.

In a group of persistently treated patients, repeatedly negative serology was more frequently associated with a monocyte-lymphocyte ratio less than 0.45, with lymphocytes higher than 1,350 per c.c., and with monocytes less than 700 per c.c.; and persistently positive serology was more frequently associated with a monocyte-lymphocyte ratio higher than 0.45, with lymphocytes lower than 1,350 per c.c., and with monocytes higher than 700 per c.c. than would be expected from random association.

The changes observed in the cytology of the treated patients as compared with the untreated patients were similar in direction and affected the same cells as the changes observed in the spontaneously regressed experimental disease as compared with the period of lesion activity. This similarity lends additional weight to deductions drawn from the experimental disease as applied to human syphilis.

ANEMIA, Aplastic, Analysis of So-Called, Thompson, W. P., Richter, M. N. and Edsall, K. S. Am. J. M. Sc. **187**: 77, 1934.

Thirteen cases of "aplastic" anemia have been studied. In all, the disease was characterized clinically by a progressive decrease in the number of erythrocytes, leucocytes and platelets, a progressive hypocythemia. In all, evidences of attempted regeneration were noted in the peripheral blood.

The marrow revealed a wide variation in microscopic appearance. In only 1 of the 13 was the marrow aplastic. In 2 it appeared hypoplastic, in 2 it was morphologically normal. In the remaining 8 there was moderate to marked hyperplasia of one or more of the formative elements.

It is felt that many of the restrictions and limitations placed on the clinical and pathologic diagnosis of "aplastic" anemia should be removed and that progressive hypocythemia alone should be sufficient reason for making the diagnosis, provided leucemia can be excluded.

The lack of correlation between the blood picture and bone marrow morphology has been discussed. It is felt that examination of the peripheral blood may be, at times, no index as to the condition of the blood-forming apparatus. Specifically, progressive hypocythemia as observed in these cases is not, in itself, sufficient evidence on which to postulate the presence of aplastic bone marrow.

This strongly suggests that it is quite possible to have serious interference with the normal processes of development, maturation and delivery of blood cells without evident alteration in the cellular content of the marrow.

B. COLI HEMOLYTICUS, Clinical Significance of, Niles, W. L., and Torrey, J. C. Am. J. M. Sc. **187:** 30, 1934.

Strains of *B. coli* possessing hemolytic properties are frequently isolated from human feces (31 to 45 per cent of stools). They are somewhat more often found and in larger numbers in feces from patients with disorders relating to the digestive tract.

There is no distinctive difference either as regards morphology or cultural characteristics in the hemolytic *B. coli* strains isolated from the stools of normal or sick people, although those from the former group have seldom exhibited any virulence.

The authors have been successful in relieving the smptoms of toxemia by autogenous vaccines. *B. coli hemolyticus* has thus been eliminated from the feces in 93 per cent of their cases. They have recurred in 3 per cent after one year or longer.

Vaccination is the only method for eliminating *B. coli hemolyticus* from the human feces which the authors have found effective.

TISSUE, The pH of Formalin—A Factor in Fixation, Burke, F. V. Am. J. Path. **9:** 915, 1934.

The following formula is recommended as superior and giving excellent results with all staining methods:

Distilled water	75 c.c.
Commercial formalin	25 c.c.
Pure pyridine	5 c.c.

BRUCELLA INFECTION, A Study of the Opsono-Cytophagic Power of the Blood and Allergic Skin Reaction in, Huddleson, I. F., Johnson, H. W., and Hamann, E. E. Am. J. Pub. Health **23:** 917, 1933.

The studies conducted on citrated blood of human beings who were known to have had undulant fever in past years and shortly after recovery, who are actively infected, or who have no history of the disease, show that the in vitro activity of the polymorphonuclear cells in whole citrate blood for Brucella is an expression of immunity to Brucella and an indication of the progress toward recovery in active infection. The absence of or a low phagocytic activity obtained in conjunction with a negative allergic skin test is evidence of susceptibility to Brucella infection. Infection in an individual is indicated by a positive allergic skin test obtained with Brucella nucleo-protein in conjunction with negative or low opsono-cytophagic activity of the whole citrated blood for Brucella.

The technic followed by the authors is described below:

Determination of Opsono-Cytophagic Power of Blood: The method adopted for determining the opsono-cytophagic power of blood for Brucella is a modification of the Leishman-Veitch technic and consists of mixing equal quantities of a citrated (0.8 per cent) fresh blood and a heavy bacterial suspension of living organisms in small Wassermann tubes, incubating at 37° C. for thirty minutes, and subsequently making spreads and staining with

Hastings stain. The addition of a definite amount of sodium citrate prevents clotting of the blood and inhibits the action of Brucella opsonins which are present in the serum of many normal individuals.

Preparation of Blood Specimen: The blood specimens are collected in 5 c.c. amounts in glass vials in which has been placed 0.2 c.c. of a 20 per cent solution of sodium citrate in physiologic salt solution. The final dilution of sodium citrate in the blood is 0.8 per cent. The test should be conducted on the specimens within six hours after collection provided they are kept in a cool place. The polymorphonuclear cells in blood disintegrate very rapidly when it is kept warm for two or three hours. The specimens should be thoroughly shaken directly before mixing with the bacterial suspension.

Bacterial Suspension: The bacterial suspension is prepared fresh each day by suspending several loopfuls of the growth from a forty-eight-hour liver agar slant culture in sterile physiologic salt solution of P_H 7. The turbidity of the suspension should give a reading of approximately 2 cm. when measured with the Gates apparatus. Suspensions of any of the three species of Brucella are suitable regardless of virulence.

The Test: Into clean small glass vials, such as are used for the agglutination or Kahn test, are placed 0.1 c.c. of the whole blood and 0.1 c.c. of the bacterial suspension. After mixing thoroughly, the vials are placed in an incubator for thirty minutes at 37° C. Certain strains of Brucella tend to become "fast" to ingestion by the cells after being transferred daily for many weeks. If a culture is being used daily for the test, it should be checked frequently for "fastness" to phagocytosis against whole citrated blood from a known immune person. Continuous agitation during the period of incubation tends to inhibit phagocytosis. Considerable sedimentation of the blood cells takes place during the incubation period. The cells should not be resuspended by shaking after the period of incubation. Directly after removing the tubes from the incubator, a small amount of sedimented cells is removed by means of a finely drawn capillary pipette to which is attached a small rubber bulb. A drop of the cells is placed at one end of a thoroughly cleaned and polished glass slide, and drawn across the slide by placing the end edge of another slide at such an angle that the spread thins out and terminates at or near the middle. In a spread of this type, most of the leucocytes may be found near the terminating edge of the spread. The blood film should be dried as rapidly as possible to prevent shrinking of the leucocytes. Rapid drying may be obtained by placing the slides in front of a small electric fan. A small heating unit from an electric heater, if attached to the front of the fan and operated simultaneously, will greatly increase the speed of drying.

Staining Spreads: The slides are placed face upward on a suitable rack and the spreads covered with 0.5 c.c. of Hastings stain. After an exposure of fifteen seconds, 1 c.c. of distilled water is added to the stain on the slide. At the end of ten minutes, the spread is gently washed from stain with distilled water and dried in front of an electric fan.

Estimation of the Degree of Opsono-Cytophagic Activity: The size of the organism in question and the marked degree of phagocytosis which occurs in cells from immunes has necessitated the employment of a different system of recording phagocytic activity from that which is commonly used in studies of this nature. In routine work, a total of 25 cells is counted in different sections of the spread and each cell is recorded as negative when no phagocytosis occurs, as slight when from 1 to 20 bacteria are seen in the cell, as moderate when from 21 to 40 bacteria are found in a cell, and marked when the number of bacteria in the cell is above 40. The bacteria are so numerous in those cells showing marked phagocytosis that it is impossible to count all of them.

It is realized that the foregoing method of measuring degrees of phagocytosis is only approximate. It was adopted after making thousands of examinations by different methods.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Influenza, With Special Reference to the Part Played by Pfeiffer's Bacillus, Streptococci, Pneumococci, Etc., and the Virus Theory*

INFLUENZA, because of its terrific morbidity and mortality, is, without doubt, one of the most important of all epidemic diseases. It is natural, therefore, that the literature concerning it has reached literally enormous proportions with little evidence of slackening in the flood of contributions.

While there has accumulated as a result a tremendous amount of information, it is questionable if it has ever before been gathered and correlated in one place.

To organize, abstract, and summarize this information is the purpose of this study, the completion of which will require two volumes.

In the present ninth volume of the Annals of the Pickett-Thomson Research Laboratories, which constitutes Part I of the Sixteenth Monograph from this source, the nomenclature, and history, as well as the clinical character of influenza, its varieties, symptoms, diagnosis, prognosis, mortality, and epidemiology are considered at length and in some detail.

In Part II, to be issued, the study will be rounded out by a consideration of the bacteriology of the complications of influenza as well as of the pathology, treatment, and prevention of the disease.

When completed, the monograph will represent a comprehensive survey of the literature of the investigations of this disease, summarizing the information contained in over 4,000 papers and present as well an account of the author's researches during the past fifteen years.

Those familiar with the previous monographs from this source will undoubtedly receive the present one with equal interest and recognize it as a contribution of undoubted value.

The rôle of Pfeiffer's bacillus is considered at length, the authors presenting as their own conclusion the belief that, while not the primary cause of true influenza, this organism plays an exceedingly important rôle in the production of the dangerous complications so commonly encountered in this disease.

They emphasize the importance and necessity for symbiotic studies in future investigations, an avenue hitherto subjected to but little consideration.

Like many others, the authors are strongly impressed by the symbiotic importance of streptococci in influenza and the fact that *B. influenzae*, streptococci and pneumococci when growing together enhance each other's virulence. They believe that the pneumococcus, while without special significance in the direct etiology of influenza, plays an important secondary rôle and directly influences the course of the infection. The evidence concerning the *Bacterium pneumosintes* they consider as yet inconclusive.

While the studies concerning the rôle of a filter-passing virus in the production of influenza are as yet incomplete and sometimes at variance, the Thomsons believe that the results of more recent work are consistent with the view that epidemic influenza in man is caused primarily by a virus infection, although further intensive study is needed.

Although space precludes any survey in extenso of this publication, enough has been said to indicate the importance of this contribution to what has been and, to a great extent is, one of the most baffling problems of medicine.

*Influenza, With Special Reference to the Part Played by Pfeiffer's Bacillus, Streptococci, Pneumococci, Etc., and the Virus Theory. By D. and R. Thomson, Pickett-Thomson Research Laboratory, London. Paper, pp. 640, 28 plates. The Williams & Wilkins Co., Baltimore, Md.

Die physikalische Seite des Blutgerinnungs problems und ihre praktische Bedeutung*

MANY are the theories that have been set up to explain the clotting of blood, many are the studies that have been made to determine the chemical processes involved. Practically, it is well known that the physical surface with which the blood is in contact has much to do with the clotting. In the live normal blood vessels the blood does not clot, in contact with the tissues and most foreign substances it does clot. However, the speed and the completeness of the clotting process vary greatly according to the surface with which the blood is in contact. Paraffin greatly delays clotting, glass produces rapid and strong clotting. A smooth, polished surface lessens clotting, a rough surface favors clotting. A substance with a low surface tension decreases clotting, high surface tension favors clotting. How can these facts be used practically? In the production of antitoxin a strong clot is desired to favor the full separation of serum; in the clinical transfusion of blood clotting is not desired. To lessen clotting, the needle with which the blood is drawn should be polished on the inside quite as well as on the outside as a rough surface favors clotting. The blood should not be allowed to touch glass as glass favors clotting by its high surface tension. A layer of paraffin on the surface of the glass lessens clotting but also lessens visibility. Lampert has found that amber lessens clotting almost as well as paraffin and is transparent. He hopes to find some substance that can replace glass entirely and practically eliminate the danger of clotting. Amber is a natural resin and now with all our synthetic resins such a hope may be realized.

Ergebnisse der medizinischen Strahlenforschung†

THIS stately sixth volume of 650 pages maintains the high standard set by the preceding volumes. Its 470 cuts are well selected and excellently printed on good paper. A wide range of subjects is covered as is shown by a few of the chapter headings: "Skin erythema and roentgen rays" by Alfred Reisner of Frankfort. This is a study of the various factors in the production of erythema, such as the quality and quantity of the rays, the effect of divided doses, season of the year, etc. It has 44 cuts and about 220 references. "The roentgenotherapy of arthritis deformans," by Günther von Pannewitz of Freiburg. This is a study based on the treatment of more than 1,500 arthritic joints and experimentally induced arthritis in animals. It has 60 cuts and about 100 references. "Roentgen diagnosis in obstetrics" by Paul H. Schumacher of Giessen. This demonstrates how helpful the x-rays can be in obstetrics. It has 65 cuts and about 500 references. "The roentgenological demonstration of diverticula in the intestinal tract" by Ludwig Bayer and Hans Pansdorf. A rather complete monograph giving the anatomy, physiology, origin, clinical significance, and treatment. It has 77 cuts and about 300 references.

Handbuch der biologischen Arbeitsmethoden‡

THIS part 412 of this mammoth work contains chapters on determination of the respiratory quotient in surviving tissues, the various forms of the Helm respiratory apparatus, measurement of the basal metabolism with the Simonson apparatus, and an apparatus for the analysis of gases from respiration chambers for men and animals.

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*Die physikalische Seite des Blutgerinnungsproblems und ihre praktische Bedeutung. (The Physical Aspects of the Clotting of Blood and Their Practical Significance.) By Heinrich Lampert. Georg Thieme, Leipzig, 1931.

†Ergebnisse der medizinischen Strahlenforschung. (The Results of the Medical Investigations of Radiation.) Band 6. Georg Thieme, Leipzig, 1933.

‡Handbuch der biologischen Arbeitsmethoden, Lieferung 412. By Emil Abderhalden. Urban und Schwarzenberg, Berlin, 1933.

Stedman's Medical Dictionary*

HOW can one review a dictionary other than by remarking on the number of new words that have been added and concluding with a sophistry on the rapid growth of the language of the medical sciences? This can be done briefly. Over a thousand words have been added since the last edition three years ago. The language is growing by at least one new word a day. But in this book one does find more, deserving of mention. It is to be found particularly in the preface to the first edition, written twenty-five years ago. In it, the author backed by his wealth of previous editorial experience classifies himself unequivocally as a purist in etymology and nomenclature. He states his reason for his general principles of selection of spelling, clearly and logically. In the earlier days of this particular dictionary this was an uphill fight, in which the editors of some of the leading medical journals offered no cooperation. However, as the author states, the general principles for which he has contended have gradually been adopted not only in the general medical literature but also in the other two outstanding medical dictionaries.

The volume is an authoritative reference dictionary, which possesses a distinct advantage in that it is still being edited by the man who started it and who has still been able to develop it along the same clear-cut lines on which it was started and without changes of policy which sometimes tend to confuse the general plan.

Handbuch der Chemotherapie†

THIS is the first part of the great work on chemotherapy which proposes to gather up and present systematically all the work that has been done so far in this great field. This first part includes only the nonmetallic organic compounds, but it runs to 650 pages. It includes such important compounds as quinine, emetine, cod liver oil, germanin, chenopodium, salicylic acid, santonine, chaulmoogra oil, and hundreds of others. For all of the important ones it gives the chemistry, the preparations, the chemical derivatives, the pharmacology, the animal and clinical experiments, the uses and extensive references to the literature. For example, after 25 pages given to other chinoline derivatives, 84 pages are given to quinine and its derivatives with ten pages of references. Emetine gets 33 pages, chaulmoogra oil and similar unsaturated oils 35 pages, etc. It is a veritable mine of information.

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*Stedman's Medical Dictionary. A Practical Medical Dictionary. By Thomas Lathrop Stedman, A.M., M.D. Editor of the "Twentieth Century Practice of Medicine" and of the "Reference Handbook of the Medical Sciences." Formerly editor of the "Medical Record." Twelfth edition, revised, illustrated, cloth, William Wood and Company, New York.

†Handbuch der Chemotherapie. Erster Teil. By Viktor Fischl and Hans Schlossberger. Fischers Medizinische Buchhandlung, Leipzig, 1932.

The Journal of Laboratory and Clinical Medicine

VOL. 19 ST. LOUIS, MO., JUNE, 1934 NO. 9

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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EDITORIAL

The Laboratory Diagnosis of Undulant Fever

THE disease first known as Mediterranean fever and now recognized as indistinguishable in its etiology from "Bang's abortion disease" in cattle, has well borne out the prophecy of Nicolle who said of it: "Mediterranean (undulant) fever is in the course of evolution and is tending to become chronic. It is a malady which, on account of its manifestation and chronicity, will become one of the most common and stubbornest diseases. . . . Mediterranean fever is a disease of the future."

The correctness of this forecast is reflected in the now relatively voluminous literature of Brucellosis, as the infections due to this group of organisms, the Brucella, is now generally termed.

Recent interest and the modern investigations of undulant fever began with the appreciation of fact, first suggested by the work of Evans[1] and the

later studies of Meyer and Shaw,[2] and corroborated by those of other workers, that the organisms in this group, whether of human, porcine, caprine, or equine variety are all alike pathogenic for man and widespread in their distribution.

Whether they are each separate species or simply varieties of a single species is still under discussion and, for the clinician, possesses practically only an academic importance.

The fact that all are pathogenic for man and responsible for clinical syndromes by no means always clear cut or readily recognized is a matter of definite clinical importance; but the problem thus presented to the practitioner is fortunately rendered relatively easy of solution through the proper utilization of various laboratory procedures which have been developed and which are now more or less generally available.

Brucellosis, or undulant fever, in man may be seen in four types: malignant, intermittent, undulatory, and ambulatory not always, however, clear-cut or readily distinguishable as the disease has a marked tendency to change its type as it progresses.

The ambulatory and undulatory forms, together with the chronic and subclinical infections, are those most often presenting diagnostic difficulties, as the disease has not infrequently been found to be the explanation of obscure periodical fevers, arthritis, and intestinal and abdominal disorders and has been confused with tuberculosis and typhoid fever.

Very early in the study of human brucellosis the value and diagnostic significance of agglutination reactions became apparent so that this method was for some time regarded as having a value equal to, if not even greater than, that of the Widal test in typhoid fever.

Many studies soon showed, however, that the clinical interpretation and significance of positive agglutination reactions with Brucella antigen suspensions were influenced by a variety of factors.

Probably no one has been more closely associated with the investigation of brucellosis, nor has become more familiar with all its phases than Huddleson, whose studies, indeed, are responsible for much of the information now available concerning this disease and who may well be regarded as one of the outstanding authorities on the subject, especially as concerns its laboratory study and diagnosis.

Huddleson's rapid slide method for the performance of the agglutination test, for example, has long since been recognized as a standard procedure and his recent monograph[3] presents in an admirable and thorough manner the present status of the laboratory diagnosis of this disease.

The procedures of value in the laboratory diagnosis of undulant fever are: the agglutination test, the allergic test, and the estimation of the degree of opsono-cytophagic activity, all of which are especially valuable when, as in the majority of instances, the detection of the organisms by culture has not been possible or feasible.

The determination of agglutinins, whether in the serum in the case of man, or in the serum or milk in the case of animals is a procedure of great utility

which, by means of Huddleson's rapid slide method may be carried out practically anywhere.

When the macroscopic test tube method is used it must be recognized that the result may be influenced by a variety of factors among which, as determined by the work of Fitch and his coworkers[4, 5] and of Henry and Traum,[6] the more important are:

1. Hemoglobin in the serum, causing nonspecific agglutination.

2. Heating serum to 56° C. or over, which lowers its agglutination titer, an effect also produced by storage of the serum.

3. Excessive antigen turbidity which tends to decrease the titer of the serum.

4. Formalin or tricresol as preservatives which retard and may even inhibit agglutination.

5. Insufficient incubation of the test.

6. Presence of agar in the antigen, which may cause nonspecific agglutination.

7. Presence of thermolabile strains in the antigen which may be agglutinated by negative serum and not by positive serum.

While the rapid method of Huddleson, devised particularly for the examination of cattle, may also be used for tests of human serum, it is important to emphasize that the results cannot be given a similar interpretation as a negative reaction, even when repeated, does not necessarily signify an absence of infection, nor does a positive reaction even in high dilution furnish proof of an active infection, as it may be the result of past infection, either clinical or subclinical, or the result of exposure to infective materials, as in the case of veterinarians, dairy and packing-house workers and the like.

The results of agglutination tests in man must always be considered in the light of the history and other confirmatory tests.

The demonstration of allergic reactions as a method of diagnosis in the human being has been studied by various workers, Burnet[7] using a culture filtrate and Giordano[8] a killed suspension.

The determination of the opsonocytophagic power of the blood as an index of brucellosis was introduced by Huddleson, Johnson and Hamann[9] and bids fair to be definitely useful.

From the correlation of all these methods very valuable and clear-cut information may be obtained which has been presented by Huddleson[10] in the tabular form (Table I).

TABLE I

AGGLUTINATION TEST	ALLERGIC SKIN TEST	OPSONO-CYTOPHAGIC POWER	STATUS TOWARD BRUCELLA
0	0	0 – 20% of cells slight	Susceptible
0	+	0 – 40% of cells marked	Infected
+	+	0 – 40% of cells marked	Infected
0	+	60 – 100% of cells marked	Immune
+	+	60 – 100% of cells marked	Immune

The application of these methods to the future study of brucellosis in man will undoubtedly clear up many obscure clinical problems and add still more to the increasing understanding of this now widespread infection.

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10. Note 3. p. 60.

—*R. A. K.*